FY 1995 ANNUAL REPORT

Biomedical Engineering and Instrumentation Program

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BIOMEDICAL ENGINEERING AND INSTRUMENTATION PROGRAM

NATIONAL CENTER FOR RESEARCH RESOURCES

NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT FOR FY 1995

Dr. Henry S. Eden, Acting Director

Edited by Barry Bowman, OD, BEIP

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10001-27 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacokinetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert L. Dedrick, Ph.D.	Chief	CHES, BEIP, NCRR
Cynthia Sung, Ph.D.	Senior Staff Fellow	CHES, BEIP, NCRR
Paul F. Morrison, Ph.D.	Physical Scientist	CHES, BEIP, NCRR
Peter M. Bungay, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR

COOPERATING UNITS *(if any)*

ETB, NIMH (J. Hsiao); CNB, NIMH (D. Pickar); PB, NCI (F. M. Balis, D. E. Cole); SNB, NINDS (J. Heiss); University of Rochester Medical Center (M. Flessner); National Naval Medical Center (C. DiGiovanni); Baylor University (S. M. Blaney)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Chemical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews

_(b) Human tissues

X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Physiological pharmacokinetic models are developed for the distribution and disposition of drugs and environmental contaminants. These models provide a plausible set of equations that can be used to extrapolate data from experimental animals to humans, and thereby improve chemotherapy and risk assessment. A pharmacokinetic model developed for topotecan in the Rhesus monkey provides a basis for design and analysis of clinical studies. Important features of the model include reversible opening of the topotecan lactone to an hydroxy acid form, and transport between the plasma and the cerebrospinal fluid. We have collaborated in the further development of a clinical protocol for the administration of AZT into cerebrospinal fluid for the treatment of AIDS dementia. Collaborative research on intraperitoneal drug administration has emphasized macromolecules. Work has been extended on the development of methods using IBZM and SPECT to quantitate D2 receptor densities, affinities, and endogenous dopamine competition for the binding sites. Analyses based on a pharmacokinetic model for IBZM suggest that data scatter from SPECT was sufficiently large that D2 receptor density and dopamine content often appeared correlated. Parameter estimation from PET ligand analysis seems preferable because of favorable counting statistics attainable with late-generation, high-efficiency gamma cameras.

RELEVANT BEIP PROJECTS: 73-159, 89-116, 91-140, 92-131, 93-103, and 95-104.

OBJECTIVES: To improve and extend mathematical models for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and humans to:

(1) Account for species differences in drug distribution.

(2) Provide a rational basis for extrapolating toxicity from experimental animals to humans.

(3) Provide a basis for optimizing chemotherapy, with particular emphasis on regional drug delivery.

(4) Enable rational transfer of *in vitro* thermodynamic and kinetic data to *in vivo* cases.

METHODS EMPLOYED: Mathematical models are developed from physicochemical, physiological, and anatomical information and the principles of chemical-reaction engineering. Resulting ordinary or partial differential equations are solved analytically or numerically and compared with experimental data. Uncertainties are clarified by additional experiments and model modification.

(1) For the treatment of leptomeningeal neoplasia, drugs can be administered directly into the cerebrospinal fluid (CSF). Clinical interest has been enhanced by the development of pharmacokinetic theory that predicts a large pharmacokinetic advantage in many cases. We collaborated in studies to characterize the pharmacokinetics of topotecan, a topoisomerase I inhibitor and potential anticancer drug. An interesting aspect of the pharmacology of topotecan is the pH-dependent, reversible ring opening of the active lactone form to an inactive hydroxy acid form. Both forms were assayed in preclinical studies in monkeys receiving injections of the drug intravenously or directly into the CSF of the ventricles. The data were analyzed with a physiologically based compartmental model, as well as with a model-independent method. CSF exposure to the active form of topotecan was 450 times higher when 0.1 mg was given intraventricularly than when 4.5 mg was given intravenously. At the doses tested, no systemic or neurologic toxicity was observed. The physiologically based compartmental model provides a basis for extrapolation to human studies.

(2) Research has continued into the development of methods using IBZM and SPECT to quantitate D2 receptor densities, affinities, and endogenous metabolite competition for these binding sites. We used a physiological pharmacokinetic model of IBZM, developed previously to account for drug metabolism and distribution in rats and humans, including the accounting of benzamide specific and non-specific binding in brain tissue. Other investigators using PET have shown that the percent of D2 receptor bound can be determined by fitting model equations to the whole tissue binding

data (of raclopride) from experiments in which dopamine is present or completely suppressed, under the assumption that D2 receptor levels remain constant in the face of dopamine change. We endeavored to determine if percent D2 bound could be estimated (1) when dopamine suppression was not complete, (2) when D2 receptor levels were not constant (e.g. as with up-regulation associated with long term exposure to D2-binding drugs), and (3) SPECT rather than PET ligands were employed.

We used the following approach: (1) to generate plasma concentration and D2 and non-D2 brain concentration time histories from the pharmacokinetic model (for any specific activity of IBZM ligand), with preassigned receptor density and affinity, non-specific binding, and total dopamine concentration parameters; (2) to scatter the labelled IBZM brain concentration values randomly (they were sampled every 3 min, according to Poisson counting statistics variation, given a maximum allowable total radiation dose of 5mCi); and (3) to back-fit only the brain-region portion of the pharmacokinetic model to these simulated experimental data, in order to determine mean parameter values and their variances. Four parameters needed to be determined from data: the non-specific binding constant, the specific binding affinity of IBZM, the D2 receptor density, and total dopamine concentration.

Results included the finding that data scatter from SPECT (mainly arising from Poisson variation secondary to poor collection efficiency through the collimator) was sufficiently great that the D2 receptor density and dopamine content parameters often appeared correlated, and disallowed unique determination of these parameters. Furthermore, the type of data collection in SPECT that might resolve this correlation depended strongly upon the absolute (and experimentally unknown) amount of dopamine present. These problems suggest that parameter estimation from SPECT data should now be abandoned in favor of PET ligand analysis, where much more favorable counting statistics can be achieved with the use of late-generation, high-efficiency gamma cameras.

(3) Dementia is commonly associated with acquired immunodeficiency syndrome (AIDS). While the human immunodeficiency virus (HIV) does not appear to infect neurons directly, cells such as microglia within the brain may be infected, and this can apparently lead to central nervous system pathology. Preliminary clinical studies from France showed that administration of AZT directly into the CSF was well tolerated in nine patients, and five of them demonstrated improvement in their symptoms. We are continuing to collaborate in the design of, and in the development of the rationale for, a Phase I clinical trial. Calculations suggest a very large and possibly exploitable pharmacokinetic advantage resulting from direct administration of AZT into ventricular CSF, but AZT removal from the CSF is relatively rapid; ventricular levels, therefore, are probably not representative of concentrations in the subarachnoid space. The depth of penetration of AZT into the brain from the CSF is a critical issue that we have examined in rats both experimentally and theoretically by means of microdialysis. Efflux from the brain of

brain of rats by a probenecid-inhibited process has led to incorporation of probenecid in the clinical protocol in an attempt to improve AZT kinetics in the CSF and to increase the depth of AZT penetration into the brain. A draft protocol for a Phase I clinical trial has been prepared, and the stability of AZT in the implantable pumps proposed for the trial is under investigation.

SIGNIFICANCE: Drugs and other chemicals are tested for effects in animals and *in vitro* systems, with the aim of extrapolating the results to humans. Both the risk associated with environmental contaminants and the optimization of therapy are at issue.

PROPOSED COURSE: To continue pharmacokinetic modeling, with consideration of pharmacodynamic and cytokinetic events and drug interactions. To continue the support of regional procedures and other measures to overcome drug resistance. To perform research designed to investigate distribution and metabolism of environmental contaminants, and to find methods for incorporating pharmacokinetics in models of risk assessment. To investigate chemical metabolism *in vitro*, in conjunction with pharmacokinetic models for quantitative prediction of metabolism *in vivo*. To extend distributed models for the description of drug movement through tissue.

PUBLICATIONS:

Blaney S, Cole DE, Godwin K, Sung C, Poplack DG, Balis FM. Intrathecal administration of topotecan in nonhuman primates. Cancer Chemother Pharmacol 1995;36:121-4.

Flessner MF, Dedrick RL. Monoclonal antibody delivery to intraperitoneal tumors in rats: effects of route of administration and i.p. solution osmolality. Cancer Res 1994;54:4376-84.

Flessner MF, Dedrick RL. Role of the liver in small solute transport during peritoneal dialysis. J Am Soc Nephrol 1994;5:116-20.

Flessner MF, Dedrick RL. Intraperitoneal chemotherapy. In: Gokal R, Nolph KD, eds. A textbook of peritoneal dialysis. The Netherlands: Klewer Academic Publishers, 1994;769-89.

Sung C, Blaney S, Cole DE, Balis FM, Dedrick RL. A pharmacokinetic model of topotecan clearance from plasma and cerebrospinal fluid. Cancer Res 1994;54:5118-22.

Bungay PM, Dedrick RL, Fenstermacher JD, Patlak CS, Krindel P. Estimation of blood sampling errors resulting from metabolism and solute exchange between plasma and formed elements. J Pharmacol Toxicol Methods 1994;32:169-75.

Bungay PM, Fenstermacher JD, Krindel P, Dedrick RL, Patlak CS. Cross-flow plasmapheresis technique for continuous ex vivo plasma sampling. J Pharmacol Toxicol Methods 1994;32:177-88.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10034-18 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Three-Dimensional Histological Reconstruction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen Leighton, Sc.D. Mechanical Engineer MES, BEIP, NCRR

COOPERATING UNITS *(if any)*

Marine Biological Laboratory (A. Kuzirian); LAS, NINDS (J. Olds)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Mechanical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews
_(b) Human tissues
X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A semiautomatic system for acquiring three-dimensional structural information about histological material is being developed. The system should be faster and more reliable than techniques that use serial sections, although resolution may be limited. In brief, an embedded tissue block will be fixed relative to a scanning electron microscope imaging system; the surface of the block will be imaged and the image stored, and successive slices will be removed by a built-in microtome. Handling and registration of thin sections will thus be eliminated. Human and computer pattern recognition will transform the resulting set of images into a three-dimensional reconstruction. The images of *Hermissenda crassicornis* obtained by this technique correlate well with TEM images of the same tissue.

PHS 6040 (Rev. 5/92)

OBJECTIVES: (1) To facilitate making schematic diagrams of neural networks. (2) To facilitate developmental studies of small organs and organisms. (3) To do three-dimensional reconstruction of biological structures.

METHODS EMPLOYED: In order to widen the applicability of Serial Block Face Imaging (SBFI), preliminary studies were done with human tonsillar tissue, both frozen and embedded in epon. Imaging was done in several modern field-emission scanning electron microscopes (FESEMs) to determine the resolution limits of the technique and to assess any other implications of modern FESEM for SBFI.

MAJOR FINDINGS: The improved FESEMs permit visualization of structures as small as the trabecular meshwork within some epon-embedded cells. It is interesting that this structure is difficult to image with traditional SEM because of its combination of fine structure and three-dimensional structure. Two major parts of the SBFI cycle are impacted very favorably by the change from traditional SEM to FESEM. The time required for oxygen etching may be greatly reduced, since higher-resolution images do not require nearly as deep an etch to show the relevant morphology. In many cases, the metal-coating part of the cycle is not required at all because the FESEM can be operated at low kV without charging artifacts. These two improvements mean that the overall cycle time can be significantly reduced. Frozen tissue was also imaged successfully, paving the way for rapid examination of unfixed tissues in three dimensions.

PROPOSED COURSE: We expect to try our system with immunogold-labeled tissue. We also expect to rearrange an OTOTO staining procedure originated by Tanaka et al. for use with SBFI. This project relates to three of BEIP's intramural research collaborations: infra-red sublimation of ice as a freeze etching technique (Dr. T. Reese, NINDS); hydrostatic tissue clamping (Dr. C. Collin, NINDS); and "en bloc" removal of soluble protein for subsequent serial block face imaging (Dr. L. Margolis, NICHD).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10039-18 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biophysical Instrumentation and Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Marc S. Lewis, Ph.D.	Research Chemist	OD, BEIP, NCRR
Peter J. Basser, Ph.D.	Biomedical Engineer	MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Microanalysis Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project is intended to develop new instrumentation and methods, and to improve existing instrumentation and techniques, for the characterization of biological macromolecules and the study of their interactions. Analytical ultracentrifugation, the techniques ancillary to it, and methods of data analysis using mathematical modeling appropriate for these techniques are the major areas of interest.

Studies have continued on the application of mathematical modeling to problems of ultracentrifugal analysis, and on the development of new methods of performing experiments and analyzing data. The 1994 report described the development of a new method of performing experiments and analyzing data for the analysis of protein-nucleic acid and protein-protein interactions. Work in this area has continued, with emphasis on developing faster and easier methods of using MLAB. Other studies have been initiated on the application of signal processing and image enhancement techniques to ultracentrifugal data analysis.

OBJECTIVES: To develop data acquisition systems for analytical ultracentrifuges; to develop appropriate software for use with these acquisition systems; to develop new methods of using new analytical ultracentrifuges; to develop new analytical methods applicable to these new experimental techniques, using MLAB software for the analysis of ultracentrifuge data by mathematical modeling techniques.

METHODS EMPLOYED: Mathematical modeling studies have involved the use of computer simulations for the development of new techniques, followed by the successful analysis of experimental data. New experimental and analytical techniques were developed that are applicable to any interacting system in which the two reactants have different absorption spectra. Typical examples are protein-nucleic acid interactions or protein-protein interactions in which one of the reactants has a chromophore modified to give a different spectrum, or in which one reactant has 280-nm absorption and the other does not. We are now working on the development of MLAB "Do" files, which should effect a marked reduction in the work of analyzing ultracentrifuge data by enabling an investigator to perform an analysis by typing in a relatively small number of "Do" commands, rather than the large number of commands presently needed. This will greatly increase the speed of analysis, reduce the possibility of error, and generally make the methods more user-friendly. The studies that have been initiated on the application of signal processing and image enhancement techniques to ultracentrifugal data analysis involve investigating the effectiveness of various standard methods for enhancing these data.

MAJOR FINDINGS: These new methods were designed to use data from the new Beckman XL-A analytical ultracentrifuge, and are dependent on the extended wavelength capabilities of this instrument. These techniques were described in detail in the 1994 report and have been extensively applied to studies described in the accompanying report for 1995 (see Z01 RR 10184-12). The work on software development and that on the application of signal processing and image enhancement methods, while promising, are still preliminary.

SIGNIFICANCE: The combination of multiwavelength scans with this new method of data analysis has provided a robust and precise method for the ultracentrifugal analysis of protein-protein and protein-nucleic acid interactions, and has greatly facilitated thermodynamic analyses of interactions that are of critical importance in molecular biology. The work currently in progress holds significant promise for further enhancing capabilities in such analyses.

PROPOSED COURSE: To continue studies of the types described above.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10097-15 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies in Cardiovascular Dynamics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Chadwick, Ph.D. Head, Biomech. Group OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

INSERM U. 120 (A. Azancot-Benisty); INSERM U. 141 (B. Levy); Laboratory of Physical Mechanics, University of Paris XII (J. Ohayon, C. Oddou); Pennsylvania State University (C. Dong)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

(a1) Minors

(a2) Interviews

(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have refined our existing model for myocardial and ventricular mechanics to include the effects of collagen on the elasticity of the myocardium and filling of the left ventricle; wall thickness and curvature effects; pathological fiber organization; myocardial blood flow; myocardial oxygen demand; effects of electrical activation patterns; interaction with the right ventricle; and dynamic vibrations. The model was used in conjunction with ultrasonic kinematic data, gated radionuclide ventriculography, and left-heart catheterization data; and implanted ultrasonic crystals were used to determine performance and contractility parameters. The physiological relationships between phasic coronary arterial, venous, and left ventricular pressure and blood flow were determined during a long diastole and under controlled conditions. A theoretical model was developed and was used to describe contraction of arterioles; it will be used to study mechanisms of hypertension. Theoretical models investigating residual stress in the myocardium and contraction with an ischemic zone are being developed. Congenital heart defects with right ventricular pressure and/or volume overload have been modeled.

OBJECTIVES: To provide a framework for determining ventricular function, both at the fiber level and for the entire structure; to relate the relationship between coronary pressure and flow to the poroelastic rheology of the myocardium; to provide a framework for evaluating those aspects of cardiovascular function that depend on the filling of, and ejection from, the left ventricle; to develop new noninvasive measurement techniques for cardiovascular research; and to provide a theoretical framework for the prediction of myocardial ischemia and the severity of congenital heart defects.

METHODS EMPLOYED: An interdisciplinary approach is used involving clinical ultrasound data, hemodynamic measurements on animals, physical models, and mathematical models.

SIGNIFICANCE: This research program, with its interdisciplinary approach, has the potential to increase fundamental knowledge in cardiovascular physiology and to improve diagnosis and treatment of cardiovascular disease.

PROPOSED COURSE: Experiments are planned with INSERM U. 141 to investigate the effects of vessel stretch by volume loading on coronary blood flow. The theoretical model will be extended to include this effect. Experiments are also being planned to determine the mechanical properties of arterioles as a function of muscle tone and transmural pressure, and interpretation will be made with the theoretical model. The theoretical model of the left ventricle is being extended to deal with regional abnormalities. This extension necessitates developing mathematical techniques to deal with nonaxisymmetric conditions.

PUBLICATIONS: Ohayon J, Chadwick RS, Azancot-Benisty A, Ito Y. Distortion du ventricule gauche et interaction ventriculaire. Comptes Rendus de l'Acad Sci Paris 1993'317(II):877-84.

Ohayon J, Chadwick RS, Herbin R. Nonuniform cavity pressure and ventricular mechanics. Mech Res Comm 1995;22:205-19.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10098-15 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laser Instrumentation for Vitreous and Cardiovascular Microsurgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Robert Bonner, Ph.D. Physicist EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
IR CB, NHLBI (S. Epstein); CB, NEI (C. Kupfer); Optical Science Branch, Naval Research Laboratory; Spectraphos; Boston Scientific, Inc.; Washington Cardiology Center

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This program initially developed laser microsurgical techniques centered on ophthalmological applications (pulsed carbon dioxide lasers and pulsed ND:YAG slit lamp-based laser systems). Its primary focus for the last seven years, however, has been in cardiological applications, in particular laser angioplasty and the development of pulsed, solid-state infrared lasers for microsurgery. We demonstrated the feasibility of transmission through flexible fiber optics of pulsed IR, visible and UV lasers, and the tissue responses to these lasers. In particular, we have measured the dependence of acoustic transients and ablation efficiency on increasing pulse fluence with catheter systems used clinically, and have concluded that acoustic transients play a dominant role in the clinical results of pulsed laser angioplasty. New IR laser sources were developed in conjunction with the Naval Research Lab and Quantronix Corp. which utilize strong water absorption and can be transmitted through low-loss, cladded optical fibers. Clinical trials were completed of peripheral and coronary laser angioplasty, using either pulsed-dye or pulsed infrared lasers with computer-controlled fluorescence guidance. Recent work has involved identification of the tissue and biological effects of pulsed lasers, particularly the large role that acoustic transients and transient, moderate (~60°C) thermal elevations play in acute and chronic clinical responses. Since pulsed laser angioplasty principally disrupts the atheroma at stenoses mechanically rather than ablating or removing it, optimization of clinical results must be balanced against increased rates of dissection.

OBJECTIVES: Current objectives are: (1) to evaluate the relative advantages of various pulsed laser sources for angioplasty (pulsed-dye, excimer, and Tm:YAG lasers); (2) to design and develop microsurgical systems using these sources; (3) to determine the proper methodology for using these laser systems; (4) to evaluate the performance of these systems both *in vitro* and *in vivo*, and in comparison with new, alternative nonlaser techniques; and (5) to examine other areas of clinical importance when using these devices, in particular the response of tissues to acoustic and thermal injury (e.g., restenosis).

METHODS EMPLOYED: Careful dosimetry of laser-tissue effects determines relative efficacy of the different laser sources. Tissue histological damage, as well as thermal transients surrounding the ablated volume, are quantified. Variation in laser pulse parameters (wavelength, temporal structure, and spatial structure) is performed in order to characterize the physical processes involved, and to understand and predict effects. A variety of physical detection methods have been used in order to evaluate effects and test models of laser damage in tissue, both *in vitro* and *in vivo*. Final evaluation of the laser system's performance is based on intravascular ultrasound examination of clinical lesions before and after therapy in conjunction with long-term follow-up.

MAJOR FINDINGS: (1) Pulsed-dye laser angioplasty was able to cross total occlusions in patients, principally by the disruptive force of acoustic transients associated with tissue and blood vaporization. (2) Blood-enhanced local absorption on/within tissue allowed success of multifiber catheters at pulse fluences below the ablation threshold observed in the absence of blood. Fluorescence spectroscopy during laser sequences confirmed the presence of thin blood layers $>50\mu m$ between ~70% of the optical fibers and the tissue. (3) A high incidence of coronary artery dissections were associated with the large acoustic transients generated by rapid expansion of vapor bubbles. (4) Laser-induced shock waves can pace ventricular contraction (although ECG-gating of laser pulses to the ventricular refractory period or high pulse-repetition rates can inhibit the abnormal pacing). (5) Acoustic transients appear to tear the annuli of stiff plaque associated with stenoses and thereby overcome their constraint of vessel lumen, thus allowing lumen expansion. However, this mode of action appears to be intrinsically prone to a high rate of dissection and is only associated with minimal debulking of stenotic lesions. In our studies and in others, pulsed laser angioplasty has been generally unsuccessful in very stiff, highly calcified stenoses. (6) New "smooth pulse" Tm:YAG (or Er:YAG) laser systems that we developed with NRL can ablate with smaller acoustic transients and may reduce the dissection rate, but their efficacy in angioplasty might also be limited. These ambitious laser coronary angioplasty systems might provide possibilities for advancements in microsurgery in a variety of other fields.

SIGNIFICANCE: Successful development of an efficacious means of relieving atherosclerotic obstructions in human blood vessels (particularly the coronary arteries) by percutaneous laser angioplasty could have a dramatic effect on the practice of cardiology and vascular surgery, if the clinical results can be improved to the extent of making this method superior to those of balloon angioplasty or other newer mechanical atherectomy devices. The Tm:YAG laser (or GaInAs laser diodes)/silica optical fiber systems and the Er:YAG laser/fluoride fiber on which we have worked appear to have numerous applications in other areas of endoscopic microsurgery. The Er:YAG has become the laser of choice for dental microsurgery following our earlier pioneering reports on its potential in such applications.

PROPOSED COURSE: Evaluation of new low-pulse energy/uniform fluence schemes for excimer laser angioplasty. Refinement of IR solid-state laser microsurgery systems; testing of their tissue effects through *in vivo* and *in vitro* studies, with evaluation of efficacy in clinical trials.

PUBLICATIONS: Douek PC, Gandjbakhche A, Leon MB, Bonner RF. Functional properties of a prototype rheolytic catheter for percutaneous thrombectomy: in vitro investigations. Invest Radiol 1994;29:547-52.

Douek PC, Leon MB, Keren G, Mintz GS, Almagor Y, Bartorelli AL, Potkin BN, Pichard AD, Kent KM, Satler LF, Bonner RF. Failure of compensatory dilation and altered plaque composition at sites of coronary artery stenoses. J Amer Coll Cardiol 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 RR 10112-15 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Microcirculatory Blood Flow by Laser Doppler Scattering

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Robert F. Bonner, Ph.D. Physicist EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
PSL, DCRT (R. Nossal); ROB, DCRT, NCI (P. Okunieff);
Vasamedics, Inc., St. Paul, MN; Laboratoire de Biorhéologie et d'Hydrodynamique Physico-chimique, Université de Paris

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed a clinically useful method (the laser Doppler instrument and its theory of operation) for clinical measurements of microcirculatory blood flow, the density of flowing red blood cells (RBCs), and mean RBC velocity. Assistance has been given to the commercialization of this methodology and to its application to clinical research. Our clinical studies have been directed toward examining normal and abnormal microvascular dynamics through noninvasive clinical studies of skin and nasal mucosa, and toward intraoperative clinical studies of muscle and of the CNS. Considerable theoretical work has been directed toward finding an adequate construct with which to interpret the physical measurements and refining the accuracy of the microcirculatory measurements. We have discovered abnormal microcirculatory patterns and responses in the skin of patients with sickle-cell disease, hypertension, certain cardiac circulatory syndromes, diabetes, and skin cancer. The microcirculatory effects of therapy are monitored with this technique, affording a better understanding of the microcirculatory components of these diseases. We are now engaged in a clinical protocol to determine if LDF measurements showing increases in skin blood flow as a function of cumulative radiation dose to the skin during radiation therapy of a variety of deeper tumors may be predictive of the general tumor and tissue radiation sensitivity of a given patient.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To develop our understanding of the human microcirculation in health and disease, and to refine the clinical utility of laser Doppler measurements of the microcirculation.

METHODS EMPLOYED: Application of the laser Doppler flow meters to various clinical protocols is based on the understanding of this technique and of the microcirculation of the targeted tissue. Because very little clinical work has heretofore been performed on microcirculatory dynamics, protocols are frequently designed to detect significant abnormalities and then to characterize them. Theoretical modeling of the signal (and, to a limited degree, of the microcirculation) facilitates the interpretation of the microcirculatory measurements.

MAJOR FINDINGS: (1) The blood volume and mean RBC velocity signals, as well as the blood-flow signals obtained by the laser Doppler method developed at the NIH, provide reproducible and clinically useful measurements. (2) A light diffusion theory and empirical optical measurements have better defined the sample volume and the absolute calibration of the measurements for a variety of tissues. (3) Myogenic vasomotion in a variety of human tissue can easily be detected and quantified. This activity is dramatically increased in a variety of diseases and may play a critical role in microcirculatory adaptation to microvascular or rheological abnormalities. (4) Reliable measurements in the central nervous system (CNS) of patients undergoing surgery can be made. (5) Skin blood-flow measurements can be used to quantify sympathetic innervation in patients with neuropathy. (6) Microcirculatory responses of tumors to photodynamic therapy have been evaluated. (7) Studies of the peripheral microcirculation in diabetics have identified a functional loss of maximal microcirculatory dilation, which may have an important role in the morbidity associated with the impairment and loss of limbs in long-term diabetics. (8) Studies of the radiation dose response variability of skin erythema in patients undergoing radiation therapy are under way to find a method of optimizing radiation therapy cumulative dosing in a diverse patient population.

PROPOSED COURSE: To pursue clinical protocols in order to establish and evaluate the contribution of microcirculatory abnormalities to a variety of diseases. To refine the theory and physical understanding of LDV in tissues in order to facilitate laser Doppler measurements in a variety of tissues and expand the application to surface imaging techniques (e.g., retinal microcirculation, skin burns, and transplanted flaps), including those accessible via endoscopy. To continue to assist commercial manufacturers in the development of accurate, reliable, and useful instruments based upon this technology. To develop automatic algorithms for digitally sampling mean flow, volume, and velocity data during a clinical protocol in order to create a reliable data base while excluding artifactual values attributable to motion or transient hemodynamic changes. We are currently evaluating the effectiveness of automatic selection of stable blood-flow values during diagnostic measurements on patients, using a variety of statistical stability indicators during real-time acquisition processing and sampling of LDF readings. Such measurement schemes show promise for increasing the clinical reliability of LDF data, particularly in the hands of nurses, technicians, and doctors who do not have extensive training in the LDF technique. In addition, we are beginning a collaboration with the University of Paris in order to further the theoretical analysis of LDF measurements of retinal blood flow (where the transparency of the surrounding tissue alters the physics of the detected photons dramatically).

SIGNIFICANCE: The clinical research studies performed in this project have provided new means of quantifying the microvascular changes (in particular, microvascular dynamics) in a variety of diseases, such as sickle-cell disease, hypertension, scleroderma, diabetes, and allergy, as well as during and following surgical and nonsurgical therapies. If the quantitation of radiation dose-response in skin predicts the particular sensitivity of both normal and cancerous tissues to radiation in individual patients effectively, then radiation doses (i.e., number of treatments) might be adjusted for each patient to improve the efficacy of therapy while minimizing the risk of associated morbidity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10122-14 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microcomputer Applications for the NIH Biotechnology Unit (Pilot Plant)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas R. Clem, Sr., BSEE   Electronics Engineer   EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LCDB, NIDDK (Y. Shiloach)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.25	PROFESSIONAL: 1.0	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with satisfying the instrumentation needs of the NIH Biotechnology Unit (Pilot Plant) using modern equipment and low-cost desktop- or laptop-sized personal computers (PCs). PCs have been acquired and installed to supply a variety of services, including process control, data acquisition, data analysis, and administrative functions. The instrumentation and process-control functions use commercial equipment as much as possible and are interfaced using standard I/O connections, such as the IEEE-488 GPIB and the RS-232 Serial I/O ports, as well as direct digital and analog I/O components in the desktop computer.

A standard data acquisition and control program has been written and is being adapted for each fermentor system in the Biotechnology Unit. By providing the same operator interface for each system, users need to learn only one command structure to be able to operate any system. Using these techniques allows changes in the parameters measured or controlled to be accomplished relatively quickly and easily. Utilizing the computational capabilities of the computer/controller allows initial selection of the operating parameters and dynamic alteration of these parameters as the process continues, thus allowing optimization of yields or detailed study of the process parameters. Adaptive control techniques have been applied to the process, and further increases in product yield are being realized.

OBJECTIVE: To increase efficiency and productivity of the Biotechnology Unit, to expand the fermentation process capability, and to develop methods for researching the fermentation process through the expanded use of inexpensive desktop personal computers and more sophisticated control and data acquisition programs.

METHODS EMPLOYED: Desktop personal computers and interface electronics (both commercial and custom-designed) are connected to laboratory instruments and to fermentation vessels via the IEEE-488 GPIB, via the RS-232, or by direct connections. Interfacing techniques are kept as versatile as practical, in order to allow system changes to be accomplished relatively easily. The control programs are written in the high-level language Pascal so that program changes can be made easily. All data are stored in a general form (ASCII) that most data analysis programs can access.

SIGNIFICANCE: Computer monitoring and control of the fermentation process provide several significant advantages over manual methods. By using the computer to make decisions based on what is occurring in the fermentation process, parameters can automatically be altered to increase yields of a product or to produce it in purer form. The computer can also perform some of the "housekeeping" tasks associated with running a fermentation process that would normally occupy an operator. Complete records of any run are saved on disk storage for later analysis. Computerized data acquisition and control allow growth rate and other parameters to be assessed under a variety of environmental conditions. The presence of a computer in the Biotechnology Unit office provides administrative support in the form of recordkeeping and text editing. This support has taken on new importance in the light of recent concern over safety and requirements for detailed logs of materials grown in the Pilot Plant.

PROPOSED COURSE: To expand the system to monitor and control additional parameters. (Larger fermentation vessels with more modern control systems, in the process of being acquired by the Pilot Plant, will be interfaced with our current systems.) To apply knowledge gained from previous work to new systems being assembled in support of the new Protein Expression Laboratory. To apply adaptive control capabilities to the other systems in the Biotechnology Unit and in the Protein Expression Laboratory. To investigate the relative merits of other growth-control techniques, such as maintaining dissolved oxygen level by feeding nutrient based on changes in measured DO_2 level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10162-13 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Wound Healing: Biology and Rheology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas L. Talbot, M.S. Mechanical Engineer ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*
SB, DCT, NCI (G. Salomon); ROB, DCT, NCI (W. De Graff)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Preliminary studies with swine models have been completed that involved stamping an indelible grid (10 cm x 10 cm) on the skin of an anesthetized swine, excising a strip of the skin (1 cm x 10 cm) out of the grid area, and approximating the incision edges with silk sutures. Photographs of the grid were taken before excision, after excision, and after suturing. These photographs are being analyzed to determine the impressed strain on wound closure, and eventually to relate this information to wound-breaking strength (WBS).

Studies based on an anesthetized rat model have been completed that relate biological and pharmacological interventions to WBS. Certain groups were treated pharmacologically during the wound-healing process. A significant decrease in WBS was observed in these groups, compared with that observed in control groups. Further studies will include the comparison of a tumor-bearing group with control groups.

PHS 6040 (Rev. 5/92)

MAJOR FINDINGS: Anesthetized rats, treated with 8 mg/kg adriamycin prior to (or on the day of) wounding, demonstrated decreased wound-breaking strength in incisional wounds at all intervals after wounding.

Decreased amounts of collagen and DNA, lower mitotic rates, and decreased cellularity were noted in wound chambers from rats treated in this manner. In both the incisional wound and the wound-chamber models, rats treated with adriamycin seven days after wounding showed a less dramatic healing impairment. No differences in collagen type were noted between chambers from the adriamycin-treated rats and those from untreated rats. The data suggest deficient quantities of platelet, macrophage, and lymphocyte factors; a decreased rate of collagen synthesis; and impaired collagen remodeling. Wound healing was examined in two wound-healing models in control rats and animals bearing methylcholanthrene-induced sarcomas, in order to evaluate experimentally the effects of tumors on wound-breaking strength. In a dorsal incisional wound model, a significant decrease in wound-breaking strength was noted from 19 days after the implantation of tumor onward. The amount of the breaking-strength deficit progressed with the size of the tumor. In a wound-chamber model, hydroxyproline levels were measured as an index of collagen content, and 3 H-thymidine incorporation was related to DNA content in order to give an index of DNA synthesis. Histology and collagen types were also examined in the wound-chamber model. The tumor-bearing state produced no significant change in any of the parameters studied in the wound chambers. The tumor-bearing state appears to inhibit wound healing in skin wounds but has no effect on healing in a model of a deeper wound. This difference in healing in the two wound models may be explained either by a cross-linking deficiency or by differential capacities for healing different types of wounds in tumor-bearing rats.

TGF-beta, now a readily available growth factor as a result of recombinant techniques, has been reported as a wound-healing enhancement agent. We ran a series of experiments incorporating our present wound model to investigate the effects of various concentrations of TGF-beta at the wound site. Paired dorsal incisions were filled with an absorbable collagen matrix, to which either 0.5 µg, 5 µg, or 50 µg of TGF-beta were added. Preliminary results indicate that TGF-beta does indeed affect wound healing, as assessed by wound-breaking strength. However, depending on the dosage, the wound-healing effect may be an increased or decreased resistance to wound bursting.

We performed similar experiments, using anesthetized guinea pigs as the experimental model, for straightforward comparison of results from previously published reports demonstrating either wound-healing enhancement or deterioration.

PROPOSED COURSE: The experimental aspects of this project have been completed.

PUBLICATIONS: Bernstein EF, Salomon GD, Harisiadis L, Talbot TL, Harrington F, Russo A, Uitto J. Collagen gene expression and wound strength in normal and radiation-impaired wounds. J Dermatol Surg Oncol 1993;19:564-70.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10184-12 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physical Chemistry of Biological Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Marc S. Lewis, Ph.D.	Research Chemist	OD, BEIP, NCRR
Peter J. Basser, Ph.D.	Biomedical Engineer	MES, BEIP, NCRR

COOPERATING UNITS *(if any)*

Center for Molecular Science, University of Texas Medical Branch (S. Wilson, D. R. Porter, A. Kumar); Randall Institute, King's College, London (M. B. Keown, H. Gould, G. Mackay); Mt. Sinai School of Medicine (J. C. Unkless); University of Nevada, Reno (P. Balagurumoorthy, R. E. Harrington); LB, NCI (S-J. Kim); LCB, NIDCD (S. Yoo); DCBDC, NCI (E. Appella, H. Sakamoto, K. Sakaguchi, N. Zambrano); IR, NHLBI (M. Bubb, E. Korn, J. Knutson); LCP, NIDDK (G. M. Clore, A. M. Gronenborn); LMB, NIDDK (R. Ghirlando)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Microanalysis Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews

X (b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the physical properties of a wide variety of biological macromolecules, with the goal of correlating these properties to the structure and function of the macromolecules. The emphasis is on the thermodynamics of the interactions of these macromolecules and on their molecular sizes and shapes. Analytical ultracentrifugation and mathematical modeling are the principal research techniques used. Studies on DNA polymerase β have involved the structure of this molecule as revealed by fluorescence and hydrodynamic behavior. Studies on chromagranin A have been directed toward investigating the thermodynamics of the interaction with the intraluminal loop of the inositol 1,4,5-triphosphate receptor of the chromaffin cell secretory vesicle. Studies on the interaction of HIV-1 RNA with nucleocapsid protein involve determining the stoichiometry and association constants that characterize the interactions, and the domains of the protein and RNA that are responsible for these interactions. Studies on p53 have been directed toward determining the relationship of the primary structure of the tetramerization domain to the thermodynamics of the self-association, and to studying the thermodynamics of the association of DNA with the DNA-binding domain. Studies on IgG binding have been directed toward understanding the interaction of cell surface glycoproteins and antibodies. Studies on polymethacrylic acid have been directed toward understanding the effect of electrostatic charge on the behavior of biologically important molecules.

METHODS EMPLOYED: Either the macromolecules have been isolated and purified from appropriate sources, or they have been prepared synthetically by conventional methods. The associations have been studied by establishing equilibrium gradients in the analytical ultracentrifuge, then analyzing the resultant data by means of mathematical modeling techniques using MLAB.

MAJOR FINDINGS:

Chromagranin A: Chromagranin A, a major protein of the secretory vesicles of the adrenal medulla, is involved in the storage of epinephrine and norepinephrine, and in the storage and release of calcium in chromaffin cells. Recent studies have involved the determination of the domain of this protein involved in the calcium- and pH-dependent monomer-dimer and monomer-tetramer aggregation. A peptide has been isolated and studied that comprises approximately one-tenth of the intact molecule, and that has the same pH and calcium ion aggregation dependency as the whole molecule. The differences between the thermodynamic parameters characterizing the peptide's interactions and those of the whole molecule indicate the role of the remainder of the molecule in influencing the interactions. Chromagranin A has also been demonstrated to interact with an intraluminal loop of inositol 1,4,5-triphosphate receptor, which has been found in the membrane of the secretory vesicle of adrenal medullar chromaffin cells.

p53 interactions: These studies have been undertaken to investigate two interrelated phenomena: p53 tetramerization, and binding of DNA. The current studies deal with the relationship of primary structure (amino acid sequence) of the tetramerization domain to the thermodynamics of tetramer formation, and with the effect of phosphorylation of serine residues on the thermodynamics of tetramerization. These studies have demonstrated the existence of a minimal core domain responsible for tetramerization, and show that the addition of appropriate sequences of amino acids toward either the C- or N-terminus of the domain enhances tetramer formation. Our studies have also demonstrated that phosphorylation has a very profound effect on tetramerization, and that it is dependent on which serine(s) are phosphorylated. Studies on the binding of DNA to the DNA-binding domain demonstrate that four p53 DNA binding domains bind to the natural p53 response elements and bend the DNA.

HIV-1 RNA-nucleocapsid peptide interaction: This peptide is a synthetically prepared 55-amino acid portion of the major HIV-1 virion nucleocapsid, and contains the active RNA-binding domain. The RNA is a 44-base pair, double-stranded segment of the non-coding 5-primer end of HIV-1 RNA. The binding is site-specific on the RNA for a stem-and-loop structure. It was demonstrated that binding to the protein induced highly cooperative RNA dimer formation, and that this dimerization is RNA length-dependent, since a 19-base pair RNA segment did not undergo dimerization upon binding to the peptide. It was also shown that binding to the first site was significantly stronger than binding to the second site for either the 44-base pair RNA or the 19-base pair RNA. It was also shown that while the binding of the first RNA was essentially size-independent, binding of the second 44-base pair RNA was stronger than binding of the 19-base pair RNA. Monte Carlo studies demonstrate the validity of the values of the association constants and give realistic estimates of their statistical uncertainties.

Interaction between the Fc fragment of human IgG1 and its low-affinity receptor: The IgG-Fc receptors are cell surface glycoproteins that bind the Fc region of antibodies and thus play a crucial role in the immune system. These studies demonstrate that this association is relatively weak, with an average free energy change of association of 7 kcal/mol for the 1:1 stoichiometry, and with a negative specific heat content, suggesting that local folding or rearrangement may be involved in the complex formation.

Measurement of molecular electrostatic forces: We have been developing new methods for measuring electrostatic and other forces between macromolecules by measuring the effects of pH and salt concentration on the concentration distribution of charged macromolecules in the analytical ultracentrifuge. New mathematical models for both transient and equilibrium sedimentation have been developed; these appear to describe molecular interactions more faithfully than did previous phenomenological models. These models offer the possibility of a better understanding of the role of nonideal interactions involving enthalpic, entropic, and electrostatic effects on macromolecules that are important in structural biology

SIGNIFICANCE: The significance of all of these studies is the same: They present information that is significant in correlating the physical properties of biological macromolecules to their structure and function. The work on DNA polymerase β and the work on RNA-nucleocapsid protein associations are particularly important, because they present new information pertaining to the role of these molecules in HIV-1 replication. Similarly, the work on p53 is important because it deals with the properties of a molecule that is critical in regulating cell growth and that is of vital interest in cancer research. The work on IgG-Fc receptors is important because it gives further information on the role of molecular interactions in the immune system. The work on molecular electrostatic forces is important because it deals with the little-known relationship between such forces and the behavior of structurally important macromolecules.

PROPOSED COURSE: It is expected that work on these molecules and others of similar interest will be continued.

PUBLICATIONS:

Kim S-J, Lewis MS, Knutson JR, Porter DR, Kumar A, Wildon SH. Characterization of the tryptophan fluorescence and hydrodynamic properties of rat DNA polymerase β. J Mol Biol 1994;244:224-35.

Bubb MR, Lewis MS, Korn ED. Actobindin binds with high affinity to a covalently crosslinked actin dimer. J Biol Chem 1994;269:25587-91.

Sakamoto H, Lewis MS, Kodama H, Appella E, Sakaguchi K. Specific sequences from the carboxy terminus of human p53 form antiparallel tetramers in solution. Proc Natl Acad Sci USA 1994;91:8974-8.

Yoo SH, Lewis MS. Thermodynamic study of the pH-dependent interaction of chromagranin A with an intraluminal loop peptide of the inositol 1,4,5-triphosphate receptor. Biochemistry 1995;34:632-8.

Appella E, Sakaguchi K, Sakamoto H, Lewis MS, Omichinski JG, Gronenborn AM, Clore GM, Anderson CW. The p53 tumor supressor protein: biophysical characterization of the carboxy-terminal oligomerization domain. In: Atassi MG, Appella E, eds. Methods in protein structure analysis. Plenum, 1995 (in press).

Balagurumoorthy P, Sakamoto H, Lewis MS, Zambrano N, Clore GM, Gronenborn AM, Appella E, Harrington RE. Four p53 DNA binding domain peptides bind natural p53 response elements and bend the DNA. Proc Natl Acad Sci USA 1995 (in press).

Ghirlando R, Keown MB, Mackay GA, Lewis MS, Unkless JC, Gould HJ. Stoichiometry and thermodynamics of the interaction between the Fc fragment of human IgG_1 and its low-affinity receptor FcγRIII. Biochemistry 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10214-11 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Photodynamic Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR

ROB, DCT, NCI (A. Russo); SB, DCT, NCI (H. Pass, W. Sindelar, M. Walther)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

_(a1) Minors

(a2) Interviews

(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Photodynamic therapy (PDT), which is the interaction of light with hematoporphyrin derivative (HPD or Photofrin II), is in clinical use in several areas of application. Currently, PDT is being used clinically for carcinoma *in situ* of the bladder and for intraperitoneal and pleural tumors. Specially designed and constructed fiber-optic probes are modified on a case-by-case basis and calibrated in terms of irradiance delivered to the tissue. Calibrated custom photodiodes are placed at strategic locations within the cavity to provide a record of irradiance values (see project Z01 RR 10272-06). Dosimetry for PDT is complex, due to the variable irradiation levels within the tissue, the sequestering of HPD, and tissue oxygenation. A Phase II/III trial has started for pleural tumor therapy.

OBJECTIVES: To evaluate PDT as a modality for treatment of selected diseases. To enhance the effectiveness of PDT as a clinical procedure by reducing the complications associated with its use. To characterize tissue, permitting theoretical analyses that predict the irradiation level at any point in the tissue distant from the point of light injection.

METHODS EMPLOYED: Two argon-ion-laser pumped liquid-dye lasers produce up to 11 W at 631 nm. 15 W can be delivered using the 514-nm line selected from the argon-ion output. All laser light is transmitted to the operating room by 600-µ fused silica fibers, to which sterile fiber-optic probes are attached. A PC-based control system was developed and installed in the operating room to control and monitor both lasers at their remote locations. A single 50-W argon-ion laser has been installed in a new surgical suite, and is being used for pleural therapy. Custom fiber-optic probes were developed from 200-µ and 600-µ fused silica fibers. Commercial sources are used when suitable probes are available.

Intraperitoneal and pleural therapy involve a wide range of tumor types. Light delivery is designed to treat all surfaces within these cavities, and similar techniques are used in both modalities. An initial surgical resection of tumor to a thickness not exceeding 5 mm is performed, and all adhesions resulting from previous surgery are removed. Two methods of light delivery are used: For the abdominal and pleural walls and organs, a wand is used that consists of an inflated tracheotomy tube filled with 0.5% intralipid solution, into which the treatment fiber(s) are inserted; for the small bowel and mesentery, a surface illumination mode is used. The wand removes all directionality of the laser light, and prevents an excessively high local irradiance of tissue. Power levels at the fiber tip are 2 kW/cm^2, which can cause coagulation of the tissue and consequential damage of the fiber. If tissue breakdown product is present on the end of the fiber, the optical energy is absorbed at the tip with generation of heat. Photodiodes are sewn into various locations in the peritoneum and pleural cavity in order to monitor the light distribution. 0.2% intralipid is introduced into the cavity to help produce uniform illumination of the surfaces. 550-nm long-pass filters are used to cover the surgical lights to reduce the PDT effect from these lights during the extensive surgery required. Treatment levels have been increased intraperitoneally to an overall irradiance of 7.5 J/cm^2 using 514 nm light, followed by irradiance of selected sites of tumor involvement using 630 nm light up to a level of 15 J/cm^2. At the power levels delivered remotely from the laser by 600-µ fiber, treatment times typically last three hours.

Intrapleural therapy is performed exclusively using 630-nm light with irradiance levels increased to 35 J/cm^2. Typical laser treatment times are two hours.

In both pleural and intraperitoneal therapies, the treatment times are dictated by photodiode sensors sewn into the cavity to indicate when the desired dosimetry level has been achieved. A PC-based computer provides both an on-line display of the irradiance levels achieved at seven strategic locations and a chronological, permanent record of the procedure on disk.

In bladder therapy, a spherically emitting fiber is used, which is positioned within the bladder (inflated to approximately 70% of full volume) with the aid of ultrasound imaging. Treatment times are typically 20 minutes long, producing a total dose of 2000 to 2500 J at 630 nm. A prototype instrument is used to position 400-µ spherical sensors against the bladder wall.

MAJOR FINDINGS: A variety of tumor types have been treated in the intraperitoneal therapy with escalation of delivered dose. Response to the therapy is assessed by clinical symptoms and by analysis of cytological washings. Phase I dose escalation studies in both modalities are complete, with the final irradiance levels indicated above.

Treatment times, even with two high-powered lasers, can reach four hours; tissue penetration is limited to a maximum of 5 mm with 630 nm light, and a maximum of 1 mm with 514 nm light. The latter, even with higher power levels available and increased photodynamic effect due to more efficient absorption, can still lead to long treatment times if substantial blood contamination is present in the intralipid medium.

The photodiode monitoring system is now used routinely to control therapy. Prime advantages of this system are its ability to account for fluctuations in laser power and for reduced irradiance due to blood contamination in the intralipid medium.

SIGNIFICANCE: Photoradiation therapy has been shown to be effective in certain instances, to have potential in others, and in some cases (e.g., pulmonary metastatic disease) to provide a unique approach to the treatment of cancer. Future acceptance of PDT will depend on several factors, some of which are addressed here. A procedure for assessing the appropriate level of irradiation is essential in designing zones of treatment. In addition, identifying responsive diseases and requisite treatment modalities is of prime importance with PDT.

PROPOSED COURSE: We will continue human clinical trials (Phase II/III), and investigate animal models as necessary to explore new approaches. Light penetration studies will be incorporated with singlet oxygen detection methods to define the levels necessary to cause the cytotoxic effects of PDT. Evaluation and interpretation of photodiode and other techniques for assessing light delivery will continue.

PUBLICATIONS: Tochner ZA, Pass HI, Smith PD, DeLaney TF, Sprague M, DeLuca AM, Harrington F, Thomas GF, Terrill R, Bacher JD,

et al. Intrathoracic photodynamic therapy: a canine normal tissue tolerance study and early clinical experience. Lasers Surg Med 1994;14(2):118-23.

Pogrebniak HW, Matthews W, Black C, Russo A, Mitchell JB, Smith P, Roth JA, Pass HI. Targeted phototherapy with sensitizer-monoclonal antibody conjugate and light. Surg Oncol 1993;2(1):31-42.

DeLaney TF, Sindelar WF, Tochner Z, Smith PD, Friauf WS, Thomas G, Dachowski L, Cole JW, Steinberg SM, Glatstein E. Phase I study of debulking surgery and photodynamic therapy for disseminated intraperitoneal tumors. Int J Radiat Oncol Biol Phys 1993;25(3):445-57.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10225-11 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Processing of High-Resolution Electron Micrographs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR
Michael Vrhel, Ph.D.	Visiting Fellow	OD, BEIP, NCRR
Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR
Philippe Thévenaz, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
CSL, DCRT (B. Trus); LPB, NIAMS (A. Steven)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the years, we have developed several computational techniques for the statistical analysis of sets of electron micrographs of biological macromolecules. These methods include various types of factorial analysis (correspondence analysis, principal components), outlier detection schemes, statistical criteria for the quantitative assessment of spatial resolution (spectral signal-to-noise ratios), and a fast algorithm for the determination of the scaling factors between two micrographs.

A current goal is to combine a larger number of micrographs with slight disparities in magnification and image contrast, in order to obtain higher-resolution 3-D reconstructions of icosahedral viruses. One objective was to address the issue of geometric corrections. This task led us to the development of new algorithms for estimating and performing the required geometrical transformations (scaling, translation, and rotation) with the least amount of distortion (least squares criterion). We are also working on the second deconvolution aspect of the problem. For this purpose, we have developed new multichannel identification and deconvolution techniques that should allow us to correct for the effect of the contrast transfer function of the microscope. These methods require the availability of multiple images of the same specimen recorded at varying degrees of defocus.

OBJECTIVES: There are currently four main objectives in this research: (1) The determination of appropriate statistical models (noise and various distortions) for high-resolution micrographs; (2) the study of algorithms for the registration of specimen views, using simple (translation/rotation) or more complex geometrical transformations; (3) the decision procedure that determines the classification of different views, as well as the detection of "bad" samples; and (4) the assessment of the quality of results obtained by averaging (e.g., signal-to-noise improvement, resolution). In particular, we have considered the problem of correcting for magnification mismatches between micrographs. Each image displays a series of independent projective views of the object under consideration. These views have different orientations and are not registered. Moreover, the micrographs may have different contrast.

METHODS EMPLOYED: The current method of compensating for small disparities in magnification is to start from an initial 3-D reconstruction, and then to compute the model projections in the directions corresponding to the current data set. Each individual virus particle is then compared to its reprojected view, and the optimal scaling factor is determined by using a spline-based least squares registration algorithm. A new 3-D reconstruction is then computed by using the scale-corrected data.

Another source of distortion is the contrast-transfer function of the microscope. We have formulated and investigated a new multichannel correction procedure. This deconvolution method enforces a projection constraint that insures an exact recovery of the signal component, while at the same time minimizing the contribution of the residual noise component. The stability of the deconvolution is improved by using multiple images recorded at varying degrees of defocus. This redundancy also provides the additional degrees of freedom necessary for noise reduction. We have also developed an approach for estimating the contrast-transfer function directly from the micrograph. In the present context, our projection-constrained strategy appears to be more appropriate than the standard statistical methods (Wiener-filter, Bayesian restoration) because: (1) the a priori knowledge required by the statistical methods is not available, and (2) the signal should not be oversmoothed in this preprocessing step. In this application, most of the noise reduction is performed a posteriori during the reconstruction phase by combining multiple independent projective views of the object under consideration.

MAJOR FINDINGS: We have applied our iterative magnification correction procedure to real electron micrographs, and were able to improve the spatial resolution of the 3-D reconstruction by nearly 10%. These preliminary results are very encouraging. We have also started performing model experiments to assess the efficiency of our multichannel deconvolution method. Our results indicate that it is much more stable and robust to noise than is the standard one-channel solution (inverse filtering).

SIGNIFICANCE: The scientific expertise we have acquired and the software we have developed should allow other scientists to use these image processing and computer reconstruction techniques to determine or understand a specimen's structure. The derived methods and the software we developed constitute an important step in obtaining higher-resolution 3-D reconstruction of macromolecules.

PROPOSED COURSE: We still need to apply both of our corrective procedures to more EM data sets, and to determine the level of improvement. For this purpose, we will also need to develop an objective resolution criterion for assessing the quality of 3-D reconstructions. We are also planning to study the feasibility of a more powerful approach for the correction of geometrical parameters (magnification, translation, and rotation) for 3-D reconstruction.

PUBLICATIONS: Unser M, Aldroubi A, Eden M. Enlargement or reduction of digital images with minimum loss of information. IEEE Trans Image Process 1995;4(3):247-58.

Vrhel MJ, Unser M. Multichannel deconvolution: the generalized sampling theory. Proc SPIE (Image Reconstruction and Restoration) 1994;2302:188-99.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10256-09 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanical Prosthetic Heart-Valve Tester

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas L. Talbot, M.S. Mechanical Engineer ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*

FDA (S. Stewart)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
   (a1) Minors
   (a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Six different manufacturers' prosthetic heart valves were tested and ranked according to the performance indices described by Swanson and Gabbay. Additionally, a technique was devised whereby the pressure drop across the aortic valve could be predicted by velocity measurements made with continuous-wave Doppler (CWD) imaging. The results of preliminary *in vitro* studies demonstrate a high correlation ($r>0.9$) between the peak velocity as measured with CWD and the predicted velocity obtained by the application of a simplified Bernoulli equation, which uses a measured maximum pressure drop across the aortic valve. Also, an acoustical signature analysis technique is under investigation for possible noninvasive detection of partially failed single-leaflet-type mechanical valves.

PROPOSED COURSE: The system works well with mechanical prosthetic valves, and the data-acquisition and analysis portions of the project have been refined considerably. Plans include the modification of the system to facilitate the testing of explanted mechanical and bioprosthetic heart valves. Additional analysis has revealed errors in measuring prosthetic-valve pressure gradients by continuous-wave Doppler, compared with standard catheter measurements. These errors appear to be systematic, and vary according to the type of prosthetic valve. No further work on this project is anticipated.

PUBLICATIONS: Stewart SFC, Nast EP, Arabia FA, Talbot TL, Proschan M, Clark RE. Errors in pressure gradient measurement in prosthetic aortic heart valves due to pressure recovery: type, size, and flow rate effects in vitro. Echocardiography 1994;11(5):425-36.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10257-09 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Propagation of Light in Turbid Biological Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert F. Bonner, Ph.D.	Physicist	EEES, BEIP, NCRR
Michael Yadlowsky, Ph.D.	NRC Fellow	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
PSL, DCRT (R. Nossal, G. Weiss, A. Gandjbakhche); LPS, DCRT; Dept. of Medical Physics, University College, London; Dept. of Physics, Bar-Ilan University, Israel

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many of the clinical research projects of the laser and modern optics group involve the interaction of light with tissue (e.g., low-coherence interferometric microscopy, diffusive-wave and time-gated optical imaging, laser microsurgery, laser Doppler blood-flow measurements, photodynamic therapy of cancer, noninvasive platelet assessment, and optical tissue oximetry). In order to quantitate these techniques more fully and to develop new quantitative, noninvasive techniques for tissue spectroscopy, we have undertaken theoretical modeling of light propagation in biological tissues and turbid media. Analytical equations have been devised to characterize various parameters of photons illuminating a tissue surface (including probability of surface re-emission at a given distance, mean path before re-emission, mean depth of penetration, and probability of absorption with depth). These expressions have been used to interpret empirical measurements on living tissues and to quantify a variety of clinical measurements (e.g., laser Doppler blood-flow and volume measurements, dosimetry in PDT of cancer, and remote sensing of tissue structure and chemical abnormalities). Recently our theoretical predictions of pathlength distributions of re-emitted photons have been applied to temporal dispersion of picosecond laser pulses in breast, brain, and muscle. Such analysis may allow noninvasive, absolute quantitation of hemoglobin and myoglobin oxygen saturation *in vivo*, as well as the concentration of a variety of important biomolecules. We have definitely demonstrated the impracticality of time-resolved optical mammography as a primary screening tool.

OBJECTIVES: To develop an insight into light propagation in turbid homogeneous and layered biological tissues, and to provide analytical expressions of general utility.

METHOD EMPLOYED: Mathematical theory of random walks on lattices and both Monte Carlo and exact enumeration computer modeling have been used to develop a quantitative analysis of light propagation in biological tissues. Empirical measurements have been obtained from tissues using laser fiber-optics systems. Comparison with diffusion theory has been examined.

MAJOR FINDING: A set of analytical equations of general usefulness when illuminating a tissue surface have been developed both in reflection and in transmission. New scaling relationships for light propagation with different anisotropy of scattering (i.e., similarity relations in analytical expressions for <cos 0>, u_s, and u_a) have been quantified. Simple relationships have been derived for resolution of time-gated transillumination imaging of deeply embedded objects, and the tradeoff between signal intensity and resolution has been evaluated for the case of embedded optical abnormalities in the human female breast. In conjunction with measurements of optical properties of normal and diseased breast tissues, our models enable us to specifically reject the practicality of optical mammography, even using time-gated techniques. We have better characterized the limits of resolution in confocal microscopy and in low-coherence interferometric microscopy with increasing depth in turbid tissues, specifically demonstrating the degradation in resolution due to coherence degradation of multiply forward-scattered light.

SIGNIFICANCE: Light is frequently used to probe and treat biological tissues; however, accurate evaluation of light propagation in tissue has been an arcane field yielding few clinically useful insights or relationships. As a result, most interpretation has been either qualitative or has required extensive correlations with techniques that have poorly controlled optical variations. Our analytical theory gives equations that have been applied and verified in a number of *in vitro* and *in vivo* applications for a variety of optical parameters of photon propagation in tissue.

PUBLICATIONS: Bonner RF, Cohen GE, Laue TM, Priezzhev AV, eds. Biochemical diagnostic instrumentation (Proc SPIE 1994). Bellingham, Washington: SPIE, 1994.

Yadlowsky MJ, Schmitt JM, Bonner RF. Coherence degradation in turbid materials probed by optical-coherence reflectometry. Appl Optics (in press).

Schmitt JM, Yadlowsky MJ, Bonner RF. Subsurface imaging of skin with optical coherence microscopy. Dermatology (in press).

Gandjbakhche AH, Bonner RF, Nossal R, Weiss GH. Absorptivity contrast detected in transillumination imaging of abnormalities embedded in tissue. Appl Optics (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10258-09 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Photochemical Inactivation of Virus and Bacteria in Blood

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert F. Bonner, Ph.D. Physicist EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

DBBP, FDA (J. C. Fratantoni); ROB, NCI

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Photochemical sterilization of blood products is the ultimate goal of this project, in which we are quantitating photochemical destruction of virus in blood and blood products and the effects of the process on the native blood components. Initial studies using an XeCl excimer laser characterized the response of virus and blood components as functions of cumulative fluence and peak irradiance. Single- and multiple-photon photochemical schemes were investigated. Single-photon photochemistry resulted in an efficacious treatment range between 10 and 20 J/sq cm, in which a hardy virus is inactivated and platelets and plasma proteins are minimally affected. Multiple-photon effects at higher irradiance led to increased protein damage in blood components, without increasing efficacy of viral inactivation. Augmentation of this therapeutic window by photochemistry (using exogenous agents such as riboflavin, tetracycline, and hemocyanin) was investigated, with a focus on the adverse effects on platelets and the relative susceptibility of different viruses, depending on the complexity of their coats. The inhibitory effect of serum albumin on photochemistry was also examined. Proposed work will examine exogenous agents that show strong affinity for binding to DNA, and thereby might increase therapeutic efficacy. The high sensitivity of blood platelets to activation and/or damage by photochemical products (e.g., singlet oxygen) has led to a renewed emphasis on optical and immunological methods of monitoring minimal lesions associated with viral inactivation photochemistry, as well as standard blood-banking techniques of preparation and storage.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To develop an efficacious means to inactivate viral and bacterial agents in blood.

METHOD EMPLOYED: Photochemical treatment, using UV-laser effects on DNA or visible light sources interacting with exogenous photochemicals. Quantitative dosimetry and assays of viral inactivation and blood component function were used to determine dose response curves. Noninvasive optical assays utilizing low-angle multiple scattering of flow-oriented platelet suspensions allowed *in vitro* determination of functional changes in platelets.

MAJOR FINDINGS: UV-laser photochemical inactivation of virus has a therapeutic window of efficacy (10 to 20 J/cm^2 @ 308 nm and $\leq$0.2 MW/cm^2) in which platelet and plasma protein function are maintained. Two-photon photochemistry at higher irradiance (~1.4 MW/cm^2) results in little improvement in viral inactivation but dramatic loss in blood component function, presumably due to peptide-bond breakage. Sensitivity of blood cells (e.g., platelets) and strong binding and/or quenching by serum albumin have proven to be severe limitations of the use of exogenous agents exhibiting non-DNA-specific photochemistry. Viral inactivation is also very sensitive to viral-coat composition and to its permeability in relation to the exogenous agents.

SIGNIFICANCE: Transfusion risks associated with the transmission of infectious agents remain a major problem in transfusion medicine. A simple, effective, noninvasive method to inactivate these infectious agents would be of enormous clinical importance. We can envision possible patient-treatment procedures using photochemical treatment of a patient's blood during plasmapheresis.

PROPOSED COURSE: Emphasis will be the optimization of the use of the blood supply, which minimizes the number of donors to which a typical patient is exposed, thereby reducing cumulative risks. A comparative study, in which new immunological methods of sensing subtle changes in platelet membrane function will be correlated with noninvasive optical assessment, seeks to elucidate and refine rapid, routine noninvasive optical analysis of blood products. A study at Georgetown University Hospital Blood Bank will examine the relationship of platelet morphology to an *in vitro* bleeding-time assay on platelets under a variety of storage conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10259-09 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Visual Target-Tracking Ability Assessment System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas R. Clem, Sr., BSEE   Electronic Engineer   EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LSES, NIMH (C. Schooler, B. Roberts)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects   (b) Human tissues   (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Abnormal eye movements found in patients with central nervous system (CNS) diseases can sometimes be revealing about the underlying pathology. Although the oculomotor nuclei, their descending primary motor neurons, and the extraocular muscles ultimately mediate eye movements, higher centers (including premotor areas in the brain stem, midbrain, subcortical areas, and cerebral cortex) are also involved in the movements. The oculomotor nuclei and neurons, together with the higher motor centers, functionally comprise five oculomotor systems responsible for different types of eye movements. Of these, the smooth-pursuit eye-movement system and the saccadic eye-movement system are of interest, for they seem to be abnormal in CNS disorders involving higher subcortical and cortical centers.

Moreover, both smooth-pursuit and saccadic eye movements have distinct and easily measured properties. Disorders of smooth-pursuit eye movements (SPEM) have been found in schizophrenia, CNS illnesses affecting the oculomotor nuclei (e.g., brainstem infarction), diffuse brain diseases (such as disorders of the Alzheimer's type), Huntington's disease, and Parkinson's disease.

OBJECTIVE: To study the ability of subjects to follow a variety of target movements, to compare the results of normal subjects with those of subjects known to have dementia, and to define a set of parameters that will characterize abnormal conditions. Tests are also being given to AIDS patients in order to assess CNS effects of HIV.

METHODS EMPLOYED: A custom interface was built to interconnect a commercial eye-position detector and a desktop PC. A second monitor connected to the PC provides the moving target: a small spot, moved horizontally in a variety of patterns. Custom programs were written to produce the desired moving-target pattern and to collect the eye-position data. These programs allow graphic display of the data and some rudimentary analysis.

SIGNIFICANCE: This system allows researchers to acquire data that were unobtainable prior to its development. The use of the PC provides the advantages of flexibility of experimental parameters, speed and accuracy of data acquisition, simultaneous acquisition of data from several sources, and storage of raw data.

PROPOSED COURSE: Improvements of data analysis and data reporting are being developed. Further tests and equipment are being developed to test a subject's abilities to coordinate eye movements with hand responses and auditory stimuli. Programs will be enhanced to allow multiple-target presentations, including moving distractor images (while maintaining the 1-millisecond sample rate of eye-position data acquisition), and to produce multiple-color displays using techniques to modify individual pixel attributes on the display. The electronic interface electronics will be modified to allow the use of the system with VGA video systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10272-08 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Photometry for Photodynamic Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
Robert F. Bonner, Ph.D.	Biophysicist	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
ROB, DCT, NCI (A. Russo, F. Sullivan); SB, DCT, NCI (H. Pass, W. Sindelar)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
(a1) Minors
(a2) Interviews

(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dosimetry for photodynamic therapy (PDT) involves measurement of hematoporphyrin derivative (HPD) concentration, oxygen concentration, singlet oxygen concentration, and therapeutic light level, all as a function of position throughout the tissue being treated (either intentionally or unavoidably). Noninvasive measurements are essentially limited to surfaces, although theoretical and empirical models can be used to estimate values some distance into the tissue. Fiber-optic probes can be used for direct subsurface measurements.

PHS 6040 (Rev. 5/92)

OBJECTIVE: To quantitate principal parameters involved in PDT in experimental models, animal trials, and human treatment.

METHODS EMPLOYED: A small handheld probe was developed to measure surface concentration of HPD. The excitation is blue light from a coherent model PRT-100 laser, electromechanically chopped at 5 Hz and conveyed to the tip of the probe by an optical fiber. The tip is conical, with an aperture 2 mm in diameter, allowing good spatial localization. Fluorescence of the HPD is separated from reflected excitation and tissue fluorescence by two filters, and sensed by a solid-state photodiode. The component at the chopped frequency is amplified and read by a digital voltmeter or, for very low-level measurements, by a lock-in amplifier. During the past year, additional experimental usage and verification have been carried out and use for other types of measurements has been explored.

For measurement of light delivered during intraperitoneal treatments, a twelve-channel, computer-based light monitoring system has been designed and built. Completely insulated solid-state photodiodes connected by coaxial cables are sutured into positions of interest within the abdominal cavity. The computer applies calibration constants for each diode and integrates the light dose at each site in real time. Multiple isolation transformers and special construction features prevent electric shock hazards.

Several new designs of photodiode assemblies have been developed and are being evaluated. A different type of interface box has been designed and built to allow integrating spheres at the ends of optical fibers to be used as the sensors. This is basically a different type of measurement, and work is under way to correlate the readings obtained by the two types of sensors. The spherical sensors are essential for monitoring light delivery for bladder cases, which are also monitored in real time.

Use of the B-3 operating room was discontinued in 1995. The apparatus from that room was therefore consolidated with the one in the second-floor operating room. A new disc backup storage system was also installed.

MAJOR FINDINGS: The HPD probe is useful for assessing the distribution of HPD and its selective concentration in malignant tissue. The light-monitoring system has been particularly useful for evaluating intralipid as a light-diffusing agent, and for quantitating the attenuation caused by the intralipid and blood. Useful information is also obtained on the light levels resulting from the operating room lights and surgeon's head lights. In addition to patient use, this system has been used in several laboratory studies to provide quantitative data.

SIGNIFICANCE: Data taken during dozens of human procedures have resulted in a substantial revision in the apportioning of light among different organs. In most instances, the real-time integrated values are now being used to control the duration of treatment. The results also clearly show the effect of leakage of blood into the intralipid, thus providing a sound basis for deciding when to change intralipid.

PROPOSED COURSE: Further software developments will make the system more versatile and easier to use. Additional theoretical and experimental work will provide a more accurate relationship between measured values and the actual effective dose.

PUBLICATIONS: Pass HI, DeLaney TF, Tochner Z, Smith PE, Temek BK, Pogrebniak HW, Kranda KC, Russo A, Friauf WS, Cole JW, Mitchell JB, Thomas G. Intrapleural photodynamic therapy: results of a phase 1 trial. Ann Surg Oncol 1994;1(1):28-37.

Sindelar WF, Sullivan FJ, Abraham E, DeLaney TF, Smith PD, Friauf WS, Thomas GF, Smith J, Okunieff PG. Intraperitoneal photodynamic therapy shows efficacy in phase 1 trial. Proc Am Soc Clin Oncol 1995;14:477.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10276-08 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microdialysis Probe Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Peter M. Bungay, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR
Robert L. Dedrick, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR
Paul F. Morrison, Ph.D.	Physical Scientist	CHES, BEIP, NCRR
Wanda R. Puckett, Ph.D.	NRC Associate	CHES, BEIP, NCRR

COOPERATING UNITS *(if any)*
ETB, NIMH (W. Z. Potter); CNB, NINDS (C. Hart); Chemistry Dept., Emory University (J. B. Justice, Jr.), Pharmacology Dept., Univ. of Texas (R. Gonzales)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews
_(b) Human tissues
X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Microdialysis probe technology provides access to tissue interstitium for either sampling of diffusible tissue constituents or delivery of bioactive substances. However, the relationship of the probe perfusate concentrations to their tissue counterparts is a complex function of solute molecular weight, solute physicochemical properties, tissue properties, probe membrane properties, probe geometry and perfusion rate, and the trauma of probe insertion into the tissue. These dependencies are being studied in order to improve the quantitative usefulness of the technology. The focus of applications is the brain in connection with diseases such as Parkinson's, AIDS and alcoholism. Endogenous solutes of interest include cyclic adenosine monophosphate (cyclic AMP) and dopamine. Exogenous substances employed as marker solutes or pharmacological agents are sucrose, acetaminophen, AZT, and ethanol. Mathematical modeling is being used to describe solute transport within the probe and in surrounding medium. Validation experiments in animals (usually rats) involve quantitative autoradiography, histology, and chemical assay of tissue surrounding the probe, as well as measurement of probe perfusate concentrations.

RELEVANT BEIP PROJECTS: 88-111, Microdialysis Probe Studies; 91-140, Intrathecal AZT for AIDS Dementia; 91-157, 94-119, Quantitative Microdialysis in Dopaminergic Tissue; 95-106, Dopaminergic Response to Ethanol in the Brain.

OBJECTIVES: The first set of general objectives is to understand and to describe more accurately the factors that affect microdialysis performance, in order to improve the usefulness of the technology and expand its capabilities. The term *performance* is used here to refer to the relationship between microdialysis perfusate concentrations and those in the medium surrounding the probe, i.e., the tissue concentrations in *in vivo* situations. A second set of objectives concerns specific applications in neuroscience research and studies of diseases such as Parkinson's and AIDS dementia.

METHODS EMPLOYED: A microdialysis probe contains a synthetic membrane, typically in a cylindrical capillary configuration. The inside of the probe is perfused with a dialysate medium. The effluent perfusate is either passed by a detector or collected for subsequent assay of the constituents that have crossed the membrane to (or from) the medium bathing the external surface of the membrane.

Unless the perfusion medium (dialysate) is allowed to equilibrate with the external medium, the concentrations of solutes in the perfusate will differ from the corresponding exterior concentrations. This phenomenon occurs because of gradients in solute concentration within the dialysate, membrane, and tissue inherent to the diffusional exchange of solute between the tissue and the dialysate. In general, empirical *in vitro* calibration of a probe cannot be relied upon in *in vivo* applications because tissue characteristics with respect to transport, binding, metabolism, vascularization, and trauma of probe insertion are difficult to mimic *in vitro*.

In this project, mathematical modeling is used to describe solute transport within the blood, tissue, and probe compartments. A variety of computerized numerical techniques are employed to simulate the microdialysis behavior from the mathematical models, in order to enable comparison with experimental observations.

The probes are characterized in *in vitro* studies. *In vivo* studies in the brains of anesthetized rats utilize model compounds to aid in our understanding of transport through tissue and the perturbation caused by the presence of the probe.

Available assay technologies include HPLC, electrochemical and spectrophotometric detection, and scintillation counting.

In addition, a quantitative autoradiography technique has been developed to determine the spatial variation in solute concentration in the tissue surrounding the probe. In this technique, radiolabeled solute is included in the dialysate flowing into the probe. At the end of a desired infusion interval, the anesthetized animal is decapitated and the head is rapidly frozen. Thin tissue slices cut perpendicular to the probe are exposed to photographic film, along with calibrated radiation standards. The concentration profile for the radiolabel is determined from the variation in light absorbance of the developed film. Both the shape of the profile and its change with duration of infusion provide information to complement measurements of the change in solute levels between the inflowing and outflowing dialysate.

MAJOR FINDINGS: General mathematical treatments for quantitative microdialysis have been developed. These treatments involve descriptions for solute transport in terms of dialysate, membrane, and external medium mass-transfer resistances. For *in vivo* applications, the description for solute transport through tissue includes the following types of processes that affect solute levels: diffusion, generation, catabolism, extracellular-to-intracellular exchange, and extracellular-to-microvascular exchange. For situations in which these processes could be modeled by zero-order or linear first-order kinetics, it was possible to obtain explicit solutions to the governing equations for transient as well as steady-state conditions. For *in vitro* applications, expressions derived for quiescent and well-stirred external media demonstrate how strongly dialysate concentration can be affected by convection in the external medium.

The explicit steady-state solution was evaluated for the sampling of tritiated water from rat brains, and the results were used to compare theoretical predictions to quasi-steady-state data from the literature. The excellent agreement obtained between theory and experimentation for the variation in dialysate effluent concentrations with dialysate flow rate provided support for the modeling approach and the predicted importance of tissue clearance processes in microdialysis behavior.

An annular flow apparatus for *in vitro* probe characterization was constructed, in which the degree of convection in the external medium can be varied in a controlled and describable manner. Studies have been performed with this apparatus, using acetaminophen as the marker solute.

Transient *in vivo* experiments were performed in which acetaminophen was administered to rats by bolus intravenous injections. In one set of experiments, microdialysis probes were used to sample acetaminophen from the caudate; in another, anesthetized rats were sacrificed at various post-injection time points to obtain brain tissue samples for assay. Good agreement was obtained between the predictions from the linear transient theory and the observed time courses of both the dialysate effluent and total tissue drug concentrations.

Thus, specific models derived within the proposed general modeling framework have been tested for both transient and steady-state probe operation. Additional simulations were performed for a variety of small solutes of interest: cisplatin, dihydroxyphenylacetic acid (DOPAC), glucose, and adenosine. The mathematical modeling efforts have been extended in several ways. One way was to develop simplified solutions in cylindrical geometry, based on collapsing the dialysate and membrane compartments into a boundary condition for the external medium compartment. Whereas the original cylindrical coordinates' transient solution was limited to low values of dialysate extraction fraction, the simplified solutions are valid over a wide range of extraction fractions. In addition, a corresponding transient solution in spherical coordinates was developed to assist in examining the importance of end effects. All of these model variations are restricted to one solute, and the extracellular and intracellular spaces have been coupled by the assumption of rapid exchange between these compartments. The various models were incorporated into MICRODIAL, a user-friendly Macintosh simulation program.

A series of *in vivo* validation experiments was conducted involving the administration of the extracellular marker [^{14}C]-sucrose through microdialysis probes into rat caudate. Extraction of solute from the dialysate was indicated by the inflow-outflow solute level difference. Autoradiography was used to follow the radial variation in tissue radioactivity. Comparisons between the measurements and the predictions from the mathematical models for both extraction and tissue profiles were consistent in suggesting that an edematous region of 35% to 40% extracellular volume fraction surrounded the probes. The edema appeared to extend beyond the approximate 3-mm-diameter interval over which the sucrose levels were detectable.

These quantitative microdialysis techniques were then used to study transport of the AIDS drug zidovudine (AZT) within the brain. These studies were undertaken in support of a proposed clinical trial for intraventricular administration of AZT to patients suffering from AIDS-related dementia. The objective was to assess whether therapeutic levels of the drug could be attained throughout the brain by this route of administration. These studies examined AZT metabolism in rat brain and the influence of systemically administered probenecid. The results implied that AZT is actively transported out of the brain parenchyma to the microvasculature, and that this mechanism is responsible for the limited penetration of systemically administered AZT into the central nervous system (CNS). Concomitantly, CNS penetration can be enhanced by inhibiting the active transport.

In the course of preparing to perform microdialysis measurements on dopamine and its common metabolites, a potential new metabolite of dopamine was identified; its production from dihydroxyphenylacetic acid (DOPAC) is catalyzed by copper and manganese ions. This new compound is of special interest, because poisoning by these two metals produces lesions similar to those observed in Parkinson's disease.

A transient model was developed for microdialysis of dopamine and the metabolites DOPAC, 3-methoxytyramine (3-MT), and homovanillic acid (HVA). The model contains intracellular compartments for free cytosolic, inactive bound, and vesicular releasable dopamine, in addition to extracellular dopamine. Nonlinear saturable expressions were employed for the extracellular-cytosolic and cytosolic-vesicular dopamine-exchange processes, and for dopamine conversion to 3-MT. Illustrative simulations for basal conditions suggest that dopamine is sampled from within 50 µm of the probe surface, while DOPAC and HVA are sampled from substantially further (500 µm) because DA is much more rapidly cleared from the interstitium than are the metabolites. In simulations for the effect of inhibition of uptake and metabolism on solute concentrations and probe performance, the model qualitatively reproduces the major features of the following experimentally observed responses: dopamine uptake inhibition by cocaine, inhibition of catecholamine O-methyltransferase (COMT) by tropolone, and inhibition of monoamine oxidase (MAO) by pargyline.

Quantitative microdialysis experiments by collaborators at Emory University indicated that the extraction fraction for dopamine could be reduced by inhibiting dopamine uptake. The mathematical model permitted the evaluation of the dopamine uptake rate constant from these measurements. The experimental dopamine extraction fraction was insensitive to inhibition of dopamine synthesis or metabolism under basal conditions, presumably because the rate constants for these processes are small compared to that for the avid uptake mechanisms.

The mathematical model was extended to predict dopamine and metabolite movement through the interstitium by the combined effects of convection and diffusion. This convection-diffusion model was used to simulate experiments in which a dopamine solution is directly infused into tissue through a cannula implanted in the vicinity of a sampling device. Considerable discrepancies were found between our simulations and experiments in which the sampling was performed either by voltammetry or by microdialysis, suggesting that infusion affects the transport of dopamine through the tissue in ways that differ from those of normal tissue.

Microdialysis studies of cyclic adenosine monophosphate (cyclic AMP) have been conducted. The evidence from the autoradiographic profiles indicates strong uptake and intracellular trapping by nonlinear saturable processes. Metabolism is involved, since HPLC separation of labeled constituents from digested tissue surrounding the probes yielded AMP, ADP, and ATP, but no detectable cyclic AMP. Detectable levels of labeled adenosine were found in the outflow dialysate samples, in addition to cyclic AMP, but no AMP was found. The studies to date are inconclusive with respect to which species are transported from the extracellular to the intracellular compartments.

Studies of *in vivo* microdialysis of ethanol were begun in connection with investigations of the effects of ethanol in the brain. Analysis of the extraction of ethanol from the perfusate of probes implanted in rat brain suggested that the lipophilicity of ethanol was an important factor in diffusion of this solute through tissue. This finding was corroborated by analysis of literature reports of ethanol microdialysis measurements in muscle of cats, rats, and human volunteers. A mathematical model for ethanol microdialysis was constructed, along with an experimental program for further model validation.

An additional beneficial outcome of the ethanol brain and muscle studies has been the development of an improved and generalized formulation of microdialysis theory. The generalization relates to a single formulation that is applicable to both hydrophilic and lipophilic solutes. In this new representation, the transport properties of the dialysate, membrane, and tissue are embodied in permeability parameters instead of mass-transfer resistances. For linear, steady-state models, the dependence of tissue permeability upon fundamental physical and geometric parameters is expressed by a simple, nondimensional relationship. This relationship provides the possibility for easy graphical or algebraic parameter evaluation from experimental measurements (for microdialysis users who do not have access to computerized analysis systems). A key parameter amenable to evaluation is the overall rate constant for solute clearance. The analysis has shown how this clearance rate constant can be obtained by algebraic calculation from the total amount of solute retained in the tissue following steady-state administration in the probe perfusate.

SIGNIFICANCE: Although originally developed for the study of neurotransmitters in the brain, microdialysis is potentially applicable to a variety of tissues and to a wide range of solutes. The technology is not solute-specific, and perfusate samples are free of macromolecules; consequently, various separation and analysis techniques can be employed in the subsequent sample processing. However, the empirical manner in which the technology has been applied has limited its utility and hampered interpretation of measurements. The mathematical models developed in this project provide a rational basis for planning and interpreting microdialysis experiments in ways that would not be feasible otherwise.

PROPOSED COURSE: The observation of substantial edema in the vicinity of acutely implanted probes should be followed up with studies investigating changes that occur over longer time intervals after probe implantation. Many investigators wait for a day after implanting probes before beginning to take measurements. The validity of their assumption that the tissue returns to normal within a day after suffering the trauma of probe insertion needs further testing.

Work will continue on the application of the dopamine model, since the results to date suggest ways in which the mathematical model and quantitative experimental techniques can be utilized together to improve the interpretation of measurements in dopaminergic tissue. The collaboration with Emory University will continue with investigations of dopamine uptake, metabolism, and receptor systems, with the principal objectives of improving the dopaminergic nerve-terminal model and extending it to include receptor-mediated regulation. The collaboration with NINDS is focussing initially on characterizing the distribution of the infusate fluid during direct interstitial infusion of dopamine solutions. Radiolabeled albumin will be added to the infusate as a slowly diffusing marker solute for quantitative autoradiography. The objective will be to improve interpretation of differences observed during infusions into rats with normal striata, compared with infusions into rats exhibiting Parkinson's-like symptoms elicited by lesioning with the neurotoxin 6-hydroxydopamine.

The global objective of studying the effect of ethanol on the brain will be pursued in the direction of quantifying changes in dopaminergic regions in response to systemic and local ethanol administration. Both the nature and the degree of the response will be assessed and related to the magnitude of local ethanol concentration. These assessments and the determination of ethanol concentration in the tissue will be obtained through combined ethanol and dopamine microdialysis measurements.

The use of a powerful finite-element software package (FIDAP) will be explored for modeling that is not feasible by analytical or previously employed numerical approaches. The modeling applications include incorporating axial as well as radial diffusion in the external medium. This will permit investigation of end effects for probes in a variety of external environments: *in vivo* and *in vitro* under both quiescent and controlled flow conditions. The influence of internal probe geometry on probe performance will also be studied.

PUBLICATIONS: Bungay PM, Morrison PF, Dedrick RL. Modeling drug delivery in the brain. In: Proceedings of the 21st International Symposium on Controlled Release of Bioactive Substances. Deerfield, IL: Controlled Release Society, 1994;105-6.

Bungay PM, Puckett WR, Morrison PF, Dedrick RL. Evaluation of kinetic parameters for dopamine and metabolites by quantitative microdialysis. In: Louilot A, Durkin T, Spampinato U, Cador M, eds. Proceedings of the 6th International Conference on *in vivo* Methods. Seignosse, France: 1994;69-70.

Puckett WR, Bungay PM, Morrison PF, Dedrick RL. Modeling dopamine pressure injection experiments. In: Louilot A, Durkin T, Spampinato U, Cador M, eds. Proceedings of the 6th International Conference on *in vivo* Methods. Seignosse, France: 1994;71-72.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10285-08 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Calorimetric Investigation of DNA/Anthracycline Drug Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*
IR NHLBI (D. Remeta)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects (b) Human tissues X (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the course of measuring the binding enthalpies of the anthracycline drug daunomycin to DNA hosts in the 10 to 20 micromolar range (where the monomeric form predominates), we found that the experimental protocol could be simplified greatly by adding a third inlet line to the existing two inlets in each channel of the microcalorimeter. This change will allow a dual-ratio mixing scheme to be used, in which we can keep the concentration of one reagent (DNA) constant and vary the concentration of the other reagent (i.e., drug) by ratioing the other two lines.

OBJECTIVE: The three-inlet-line design will allow us to vary the drug concentration as we measure the binding heats of daunomycin to a variety of DNA hosts. This approach will allow us to investigate the relationship between the drug concentration and its binding affinity to the DNA hosts directly. The binding enthalpies are good indicators of the drug's effectiveness in chemotherapy.

SIGNIFICANCE: The determination of daunomycin's binding affinity at therapeutic levels is extremely important because of the drug's toxic side effects. At present, daunomycin is the drug used second most often in chemotherapy.

PROPOSED COURSE: The three inlet mixers have been fabricated. The commercial source for hardware and software to drive the three motors did not perform as expected and has required considerable software and hardware revision in order to accomplish dual-ratio mixing at a half-step mode. We are now evaluating the calorimeter.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10296-08 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experiments With a High-Resolution Field-Emission STEM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

LN, NINDS (S. B. Andrews); LSBR, NIAMS (F. Booy); Lehigh University (J. Hunt)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Scanning transmission electron microscopy (STEM) has been combined with parallel electron energy-loss spectroscopy (EELS) to develop new methods and experiments that complement conventional electron microscopy-based structural biology. To assess the potential of valence EELS for imaging the distributions of biological compounds in cells, high-resolution (0.5 eV) spectra have been recorded from specimens of protein, carbohydrate, nucleic acid, and lipids, as well as from vitrified water. These spectra, which were obtained at low electron dose, had charateristic fine structure in the energy range 4-12 eV, corresponding to absorption maxima in the vacuum ultraviolet spectrum. Knowledge of these spectra are important for quantifying water distributions in frozen-hydrated cryosections of tissue in the STEM. Further measurements have also been performed to determine the effects of radiation damage on the valence EELS spectra. Radiolysis of frozen-hydrated specimens subsequent to damage in the electron beam has been found to result in the formation of hydrogen bubbles. Improved methods have also been developed for quantifying EELS core edge spectra recorded in the difference mode from specimens containing very low concentrations of important elements such as calcium and phosphorus.

OBJECTIVES: To investigate new high spatial-resolution and high energy-resolution applications of EELS in the field-emission STEM. To develop further parallel-EELS mapping techniques for obtaining elemental distributions in subcellular compartments of rapidly frozen, cryosectioned tissue and macromolecular assemblies.

METHODS EMPLOYED: The EELS data were obtained with a specially adapted magnetic sector spectrometer equipped with a photodiode array parallel detector. Specimens of protein (bovine serum albumin), carbohydrate (sucrose), and nucleic acid (DNA) were prepared by rapidly freezing aqueous solutions and then cryosectioning. A mixture of commonly occurring membrane lipids was prepared by placing drops of the purified extracts dissolved in chloroform onto EM grids and allowing them to dry. The field-emission source of the STEM provided incident electrons of 0.5 eV energy spread, defined by the full-width at half maximum of the zero-loss peak, as measured by the spectrometer. Spectrum-images containing ~2000 pixels were acquired at a dispersion of 0.1 eV/channel from 5- to 10-mm-sized regions of the specimen. The probe was defocused to ~200 nm to avoid undersampling, and the dose on the specimen was less than 100 electrons per square nanometer. Spectra were processed (i) to subtract the dark-current, (ii) to align the zero-loss peak, (iii) to deconvolute by the detector point-spread function, (iv) to derive the single-scattering distribution by logarithmic deconvolution, and (v) to correct for the support film where necessary.

MAJOR FINDINGS: Spectra from each group of compounds investigated had characteristic fine structure in the energy range from 4 eV to 12 eV. Two peaks are evident in the spectrum from DNA at 4.9 eV and 6.7 eV that can be attributed to excitation of pi-states in the pyrimidine and purine rings of the nucleic acid bases. The peak at 6.7 eV in the protein is due to excitation of pi-states occurring in the peptide bonds, as well as to aromatic side groups. In the lipid the pi-peak is much weaker because of the predominance of saturated carbon atoms. Sucrose contains no unsaturated bonds; therefore, no such peaks are evident. Parallel collection of these spectra over an energy range of ~80 eV has enabled us to compare the EELS data with optical measurements extending into the vacuum ultraviolet (VUV) energy region. The consistency between the electron-scattering and VUV measurements demonstrates the quantitative reliability of the EELS data. Use of valence-EELS spectra to characterize distributions of compounds in cells is limited by radiation-induced changes in fine structure. Lipids are particularly susceptible to damage, which we attribute to loss of hydrogen from saturated hydrocarbons, even at doses below 1000 electrons per square nanometer. Changes in fine structure are less evident in the other compounds at this radiation exposure.

SIGNIFICANCE: In conclusion, efficient parallel EELS spectrometers can provide distributions of certain compounds in cells. We have already demonstrated that water can be imaged quantitatively at a resolution of approximately 50 nm in frozen cryosections, but this requires knowledge of the valence EELS from the biological compounds that we obtained in the work described above. By using these reference spectra, it may also be possible to quantify distributions of nucleic acid and protein in nuclei, or lipid and protein in membranes, at around 100-nm resolution.

PROPOSED COURSE: To incorporate a slow-scan, charge-coupled device detector system in the EELS spectrometer in order to provide higher detective quantum efficiency. This improvement will help to optimize the collection of chemical information from biological specimens.

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Hug G, Leapman RD, Jaouen A. A study of EELS fine structure in three chromium carbides. In: Proceedings of the 29th annual MAS meeting, 1995 (in press).

Newbury DE, Leapman RD. The analysis of nanoscale particles by analytical electron microscopy. In: Proceedings of the 29th annual MAS meeting, 1995 (in press).

Leapman RD, Andrews SB. Improved method for quantifying EELS difference spectra from biological specimens. In: Proceedings of the 29th annual MAS meeting, 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10303-07 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assessment of Scratching in Biliary Cirrhosis Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas Talbot, M.S.	Mechanical Engineer	ACES, BEIP, NCRR
Joseph Schmitt, Ph.D.	Staff Fellow	ACES, BEIP, NCRR
Elijah Walker, M.S.	Chief	ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LDS, NIDDK (N. Bergasa), OD, NIAID (D. Alling)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects
  (a1) Minors
  (a2) Interviews

_(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with primary biliary cirrhosis suffer from severe bouts of itching, which is manifested by chronic scratching. In order to evaluate the efficacy of experimental drugs that may relieve the itching, it is necessary to quantify the scratching. A piezoelectric foil cemented to the patient's primary scratching fingernail transduced mechanical vibrations, generated by the scratching, into an electrical signal applied to the input of a miniature FM transmitter. After the signal was obtained with an FM receiver, it was processed by a custom-designed frequency counter, which in turn was interrogated and reset periodically by a personal computer. The study consisted of measurement periods of six to eight hours, both before treatment and after treatment with specific drugs. Each patient served as his or her own control.

MAJOR FINDINGS: A scoring scheme was devised and implemented that provides an average scratching activity index per hour. This index was used in a large double-blind clinical protocol to determine the viability of Naloxone, an opiate receptor antagonist, as an ameliorating agent in the treatment of pruritus. The study, which is now complete, ran for four consecutive 24-hour periods, with two control (placebo) periods and two Naloxone periods. Neither the patients nor the investigators were aware of the contents of the intravenous infusate during the study. The results clearly demonstrated a statistically significant reduction in scratching activity while the Naloxone was being infused, indicating that the drug is effective in ameliorating the pruritus associated with cholestasis. The results of this study have been published. In addition, a major double-blind clinical trial is nearly complete that will determine the efficacy of the oral medication Nalmafene, the oral counterpart of Naloxone, in the amelioration of pruritus. This study also incorporated a device that attached to the patient's finger in a splint-like fashion. This device provided a mechanism to normalize the stiffness and attachment modality of the piezo film scratch sensors. This normalization is particularly important because the protocol required the monitoring of the scratching at specific times during the various phases of the drug-control or placebo-control regimens. Parallel analysis of certain underlying circadian rhythms that may unveil imprinted scratching behavior is also under way. Preliminary indications imply that scratching may be an independent biorhythm, as well as a manifestation of a dermal antagonist.

PROPOSED COURSE: The clinical trials are complete. I may continue work on this project in a slightly different capacity, as a consultant on a research grant. The purpose of the grant is to develop the scratch-assessment instruments into a portable device that would be suitable for long-term outpatient monitoring of pruritic behavior.

PUBLICATIONS: Bergasa NV, Alling DW, Talbot TL, Swain MG, Yurdayin C, Turner ML, Schmitt JM, Walker EC, Jones EA. Effects of Naloxone infusions in patients with the pruritus of cholestasis: a double-blind controlled trial. Ann Intern Med 1995 (accepted for publication).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10305-07 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CC Image Management System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ronald L. Levin, Sc.D. Biomedical Engineer MES, BEIP, NCRR

COOPERATING UNITS *(if any)*

MIS, CC (T. Lewis)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Mechanical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to facilitate the viewing and analysis of biomedical images, the Clinical Center (CC) is in the process of designing and developing an Image Management System (IMS) to complement the current text-based Medical Information System (MIS). This new system will consist of the following four key components: (1) several different types of medical image gateways (MIGs); (2) an image archival and retrieval system (IARS) that has an MIS interface; (3) several different types of workstations (WS) possessing a common biomedical shell; and (4) an interconnecting communications network. The MIGs will permit digital radiological images (i.e., MR, CT, PET, etc.), nondigital radiological images (i.e., plane-film X ray), and nonradiological images (i.e., video-microscopic images, EKG tracings, patient monitor information, etc.) to be converted to a common format, compressed in a loss-less manner, and automatically sent to the IARS. The IARS will permit not only the long-term archiving on optical media of the images coming from the MIGs, but also the retrieval of multiple-modality images to local workstations in response to queries via the existing MIS data base, the new IMS data base, or individual departmental data bases. In the workstation area, we will be developing two or three different types of biomedical workstations. The key to this series of workstations is that they would all use the same biomedical shell (i.e., user interface). Application programs for diagnostic imaging, stereotaxic surgical planning, radiation therapy planning, tumor staging, etc., will also be developed.

OBJECTIVES: To facilitate the exchange of medically related images by permitting images from multiple sources to be accessed via a single data base and in a single file format, and to facilitate the manipulation and comparison of medically related images together with text-based medical records through the use of a specially designed biomedical workstation.

PROPOSED COURSE: During FY 95, MRIPS successfully placed "on-line" the Picker MR scanners, the IMATRON FastCT scanner, and the GE MR and CT scanners of the DRD/CC, BEIP/NCRR, and LDRR/OD. All data from these machines are transferred automatically and without operator intervention into the MRIPS Data Registry. This corresponds to over 4 GIGAbytes of data per day.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10313-07 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

High-Speed Multichannel Spectrophotometer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
Thomas J. Pohida, MSEE	Electronics Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LCB, NHLBI (R. Hendler); PCB, DCRT (H. Fredrickson)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of commercial multichannel spectrophotometers using charge-coupled devices have recently been developed for general use in biomedical research. We have explored the possibility of improving both the speed and the accuracy of such instruments by exploiting other new devices. A massively parallel approach was implemented which achieved the design specification of 100,000 samples per second with 12-bit resolution.

OBJECTIVES: To develop a 92-channel spectrophotometer capable of sampling all channels at a maximum rate of 100,000 samples per second with 12-bit resolution.

METHODS EMPLOYED: A commercial spectrophotometer will disperse the light to two 46-element photodiode arrays. Each channel will integrate the signal between sample times, digitize it, and store it in a first-in, first-out register prior to digital multiplexing for transmission to a computer. The computer will provide full control over the sampling program, as well as over such experimental parameters as flow and flash photolysis; it will also handle all of the processing and permanent storage, will display data, and will perform various other functions.

SIGNIFICANCE: Achievement of the proposed specifications has opened up a variety of new applications. The increased temporal resolution permits investigation of early events in biochemical reaction kinetics that have been impossible to measure before. The ability to follow these processes with high accuracy and at multiple wavelengths enables investigators to track individual components within a complex reaction simultaneously.

PROPOSED COURSE: Design and construction are complete, and the instrument is in regular use. We are exploring the possibility of cooling the diode arrays, adding a programmable offset to each channel, and increasing the gain to provide improved resolution. We are also looking into the possible application of image intensifiers to extend the short-wavelength response of the instrument. A new commercial stopped-flow apparatus is being integrated into the system, and computer control of experimental parameters has been extended and made more user-friendly.

An invention report has been filed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10315-07 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Model of Magnetic Stimulation of a Nerve Fiber

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bradley J. Roth, Ph.D.	Senior Staff Fellow	MES, BEIP, NCRR
Peter J. Basser, Ph.D.	Biomedical Engineer	MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
MNB, NINDS (L. Cohen, M. Hallett); Dept. of Neurology, SUNY, Brooklyn (P. Maccabee); Dept. of Biomedical Engineering, Case Western Reserve University (S. Nagarajan, D. Durand)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews

(b) Human tissues

X (c) Neither

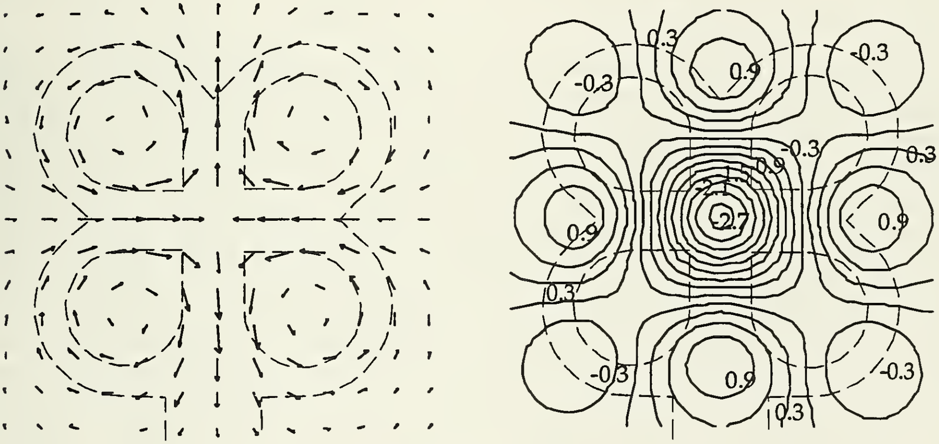
SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A model is presented to explain the physics of nerve stimulation by electromagnetic induction. Maxwell's equations predict the induced electric field distribution produced when a capacitor is discharged through a stimulating coil. A nonlinear cable model, containing active Hodgkin-Huxley elements, describes the response of the nerve fiber to this induced electric field. It is shown that the nerve fiber is stimulated by the gradient of the component of the induced electric field parallel to the fiber, which hyperpolarizes or depolarizes the membrane and may stimulate an action potential. Once the coil's position, orientation, and shape are given and the resistance, capacitance, and initial voltage of the stimulating coil are specified, this model predicts the resulting transmembrane potential of the fiber as a function of distance and time. Finally, it predicts complicated dynamics, such as action potential annihilation and dispersion. The model has been verified experimentally in humans. A new four-leaf coil design for magnetic stimulation of peripheral nerves has been developed and has been tested using *in vitro* experiments.

OBJECTIVES: To increase our understanding of the physics of magnetic stimulation, and to design improved coils, by developing a mathematical model of the interaction of a nerve fiber with the induced electric field. Also, to develop new coil geometries for use during magnetic stimulation.

SIGNIFICANCE: Although the electric field induced in an arm can be calculated accurately, its interaction with a peripheral nerve had not been elucidated. Basic questions (such as the location of stimulation) must be resolved before magnetic stimulation can be fully utilized as a clinical tool. This model provides insight into the interaction of the electric field with the nerve, and provides a tool for developing new coil designs.

MAJOR FINDINGS: The magnitude and time course of the gradient of the component of the induced electric field parallel to the nerve fiber determines if and where stimulation occurs. The location of the virtual cathode is sensitive to coil placement. A secondary virtual cathode and anode pair are predicted to exist for a circular coil. A four-leaf coil design is particularly appropriate for peripheral nerve stimulation, and its usefulness has been demonstrated experimentally.



The calculated electric field (left) and the derivative of the x-component of the electric field, dE_x/dx (right), produced by a four-leaf magnetic stimulation coil (dotted outline). The x-direction is horizontal, and dE_x/dx is given in units of mV/mm^2.

PROPOSED COURSE: New coil designs will continue to be developed and tested. The model will be extended to account for curving fibers, axon termination, and effects of the surrounding fibers in the nerve bundle.

PUBLICATIONS: Nagarajan SS, Durand DM, Roth BJ, Wijesinghe RS. Magnetic stimulation of axons in a nerve bundle: effects of current redistribution in the bundle. Ann Biomed Eng (in press).

Roth BJ. Mechanisms for electrical stimulation of excitable tissue. Crit Rev Biomed Eng (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10318-07 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Apple Macintosh-Based Image-Processing Workstation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR
Chulhee Lee, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Personal computers (such as the Macintosh) offer very cost-effective hardware configurations for simple image-processing tasks. However, programming them using conventional techniques is notoriously difficult, due to the high level of sophistication of the user interface. The main purpose of this project is to devise powerful development tools to facilitate the development of specialized software for such workstations. The first step was to create a generic image-processing shell that is easy to modify for creating specialized applications. The main feature is that all complex interactions with the user interface are handled automatically; the programmer need only be concerned with the application-specific procedures. Over the years, we have developed a comprehensive set of basic image-processing modules that perform various tasks, including filtering, multiresolution and wavelet decompositions, edge detection, point transformations, and binary operations; most of these modules are now available in both FORTRAN and C. More recently, we have begun the development of an improved, second-generation image-processing system, called Flexible Language-based Image-Processing System II (FLIPS II), that uses a powerful interpretive language and integrates most of the available subroutines. This year, we have improved the portability of the software which is now also available on a UNIX environment.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To develop an extensive software library that can be used to facilitate and standardize the development of user-friendly image-processing programs on the Macintosh computer family, as well as on more powerful UNIX workstations.

METHODS EMPLOYED: Our image-processing software library is written in C. It includes a relatively large selection of point operators (histograms, nonlinear transformations, simple arithmetics, statistics, etc.), neighborhood operators (moving average and Gaussian smoothers; Laplacian, convolution, median, and morphological filters; adaptive least squares; and B-spline filters), wavelet transforms and image pyramids, and binary operations (automatic thresholding, thinning, expansion, erosion). There is also a collection of input/output subroutines for reading and writing images in analyze or TIFF format, display, and interactive specification of points, lines, or regions of interest. Our general image-processing system, Flexible Language-based Image-Processing System (FLIPS), is centered around a powerful interpretative language that offers high-level programming capabilities and uses command variables to exchange information between modules. The basic entities that are created or modified can be whole sequences/arrays of images or volumes.

MAJOR FINDINGS: This new programming environment has shortened development time substantially while improving reliability. FLIPS has been improved considerably. This system integrates most of the currently available subroutines and facilitates the handling of both integer and real-image data.

SIGNIFICANCE: The availability of efficient development tools is essential to setting up small collaborative projects with other researchers in the NIH community. There are several initial advantages to designing an image-processing system based on a personal computer. First, this type of computer is now widely available for a variety of personal-computing tasks; therefore, the purchase of new equipment is usually not necessary. Second, the Macintosh is a very cost-effective alternative to conventional image-processing systems. Third, all Macintosh programs are designed to be very user-friendly, thus facilitating their dissemination. Finally, most data formats are standardized, which greatly facilitates the exchange of information among different applications. Custom programs and general-purpose applications (statistics, graphs, etc.) can be combined very efficiently to solve a specific problem. A recent improvement is that UNIX versions (Dec-Alpha, Sun, Silicon Graphics) of our software are now available. Hence, one can also use higher-performance workstations for performing more demanding image processing (e.g., 3D data sets, volume registration, etc.).

PROPOSED COURSE: We will extend the present environment by adding new image-processing functions. We will also improve the portability of our software to make it more usable in a UNIX environment and to facilitate integration with the MEDx system.

PUBLICATIONS: Unser M. Multigrid adaptive image processing. In: Proceedings of the IEEE International Conference on Image Processing. Washington, DC: IEEE, 1995 (in press).

Unser M. Texture classification and segmentation using wavelet frames. In: Proceedings of the IEEE International Conference on Image Processing. Washington, DC: IEEE, 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10322-07 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Acquisition System for an Ultrahigh-Resolution Dedicated STEM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carol R. Swyt, M.S. Physical Scientist OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

CAC, NIST (R. Myklebust)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An automated data acquisition system is under continuing development for the Vacuum Generators HB 501 ultrahigh-resolution dedicated scanning transmission electron microscope (STEM) to overcome the limitations of commercial systems for concurrent data acquisition and processing algorithms. As part of the new system, a data acquisition and analysis program, called Desk Top Spectrum Analyzer and X-Ray Database (DTSA), has been developed for the Macintosh II (and higher) class of Apple computers. Data acquisition is accomplished with a specially modified, commercial, NuBus-compatible I/O processor and custom-designed acquisition and control software routines. A patent (U.S. Patent No. 5,299,138), held jointly by the NIH and the NIST, was issued for this analytical tool in March 1994. The DTSA is available from the Office of Standard Reference Data (OSRD), National Institute of Standards and Technology, for a modest fee. A copyright has also been obtained by the OSRD.

METHODS EMPLOYED: The software is written in MPW Pascal for the Macintosh II and higher series of Apple computers. The user interface utilizes the full range of Macintosh utilities, including windows, menus, dialogs, and color display. Algorithms for all of the relevant physics for electron beam-excited generation of X rays in a thin or bulk specimen, and detection by a lithium-drifted silicon detector, are provided in the program. The user may select from a number of the most widely used cross sections for X-ray generation, and may input microscope, specimen, and detector characteristics that determine the final spectrum distribution. Any of the functions calculated, such as the continuum distribution and characteristic peaks before and after convolution with the detector function, may be displayed in one of the ten real-valued overlaid "spectrum displays." The information in the X-ray database is available to the user via a menu that provides relative intensities with the lines, edges, or satellites specified in the user-selected units of eV, mm, Å, or (for wavelength-dispersive X-ray spectrometry) sin(θ) for the crystal spectrometer chosen. Both linear and nonlinear spectral peak unraveling methods are available, and quantitation schemes for thin and bulk biological, geological, and other specimens are available. X-ray spectra acquired by wavelength-dispersive X-ray spectrometry may be imported, converted to energy display, and processed using any algorithm in DTSA. Among the other routines provided are qualitative analysis, spectrum calibration, beam-energy calculation based on the Duane-Hunt limit, peak stripping, background subtraction, Fourier transforms, spectral smoothing, and simple scaling and summing of distributions. Both energy-dispersive and wavelength-dispersive X-ray spectral data acquired with other systems may be transferred to the DTSA program and converted to internal format for processing. This is now accomplished using a "plug-in" code selected by the user to accommodate the spectrum file format of his acquisition system. Any displayed distribution may be sent directly to a printer or stored in a file. Menus are provided for supplying full notebook annotation of data files. The results of spectral unraveling and quantitation may be reported in any one of the common spreadsheet formats for statistical analysis and plotting, and may be output to hard copy with full file annotations.

An interface to the acquisition control software is provided in the program. The parameters for acquisition (such as data collection time, characteristics of the detector system, etc.) are supplied via the DTSA program to the acquisition software. Information such as dead time, real time, and data input rate are constantly displayed. As data are acquired, they are transferred to the Macintosh memory and are available for constantly updated display as a spectrum on the Macintosh screen and for processing with any algorithm in the DTSA program.

Version 2.2 of the program will be available in September 1995 from the Office of Standard Reference Data, National Institute of Standards and Technology. A number of commercial X-ray analyzer manufacturers have negotiated either to offer the DTSA as an option with their systems or to utilize the X-ray database in their own analyzer systems.

SIGNIFICANCE: Although it was designed for the EBIMS VG HB 501 STEM, the DTSA provides analysts in any laboratory with an easily customized means of obtaining X-ray data, spectra, or images from any electron microscope equipped with the appropriate detectors. The cost is about one-tenth that of traditional energy-dispersive X-ray systems. In addition, it provides the full capabilities for data analysis developed over the years in the EBIMS group, and those for data presentation and statistical analysis developed for the Macintosh computer. It is the only analyzer system to provide the means with which to apply these capabilities to data acquired with wavelength-dispersive X-ray analysis systems. These capabilities are implemented in the typically user-friendly Macintosh environment. The program provides the analyst with a set of tools to simulate, analyze, and better understand the generation and detection of X rays from a sample, and therefore to design more informative and efficient experiments. This work can be done on the desktop, freeing the electron-beam instrument for productive data acquisition.

PROPOSED COURSE: The code will be recompiled in Language Systems Pascal for the PowerMac series of personal computers. Image acquisition and processing routines will be developed. Modification and adaptation for the DTSA of methods developed in the EBIMS Group for electron energy-loss spectrum processing will continue. Peak fitting routines will be modified to allow the omission of some lines for elements to fit in order to optimize the processing of spectra from thin biological sections. An absorption correction will be added to the Cliff-Lorimer type quantitation algorithm to improve accuracy for spectra acquired in the NIH/NIST Nanometer Resolution Facility. In addition, negotiations will continue between commercial manufacturers of electron microscopes and X-ray analytical equipment and the NIST and the NIH for the rights to distribute and use the program.

PUBLICATIONS: Swyt CR. Comparison of simplex and multiple linear regression peak fitting using generated spectra. In: Etz E, ed. Microbeam Analysis - 1995. New York: VCH Publishers, Inc., 1995 (in press).

Swyt CR. A study of systematic errors in multiple linear regression peak fitting using generated spectra. In: Williams DB, Goldstein JI, Newbury DE, eds. X-ray spectrometry in electron beam instruments. New York: Plenum Press, 1995;159-66.

Newbury DE, Swyt CR, Myklebust RL. "Standardless" quantitative electron probe microanalysis with energy dispersive X-ray spectrometry: is it worth the risk? Anal Chem 1995:67:1866-71.

Swyt CR. Estimating systematic errors for Ca in multiple linear regresssion peak fitting using generated spectra. In: Friel J,

ed. Proceedings of the 28th annual meeting of the Microbeam Analysis Society. New York : VCH Publishers, 1994;113-14.

Newbury DE, Myklebust RL, Swyt CR. The use of simulated standards in quantitative electron probe microanalysis with energy dispersive x-ray spectrometry. In: Friel J, ed. Proceedings of the 28th annual meeting of the Microbeam Analysis Society. New York: VCH Publishers, 1994;231-2.

Newbury DE, Swyt CR, Myklebust RL. Standardless quantitative electron probe microanalysis with energy dispersive x-ray spectrometry: is it worth the risk? In: Friel J, ed. Proceedings of the 28th annual meeting of the Microbeam Analysis Society. New York: VCH Publishers, 1994;229-30.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10324-07 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Hemodynamic Models for Cardiovascular Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert J. Lutz, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR
Travis Jones, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR

COOPERATING UNITS *(if any)*

IR CB, NHLBI (J. Peacock); ROB, DCT, NCI (A. Epstein); LDDR, OD (W. Pritchard); Georgetown University Hospital (S. Lossef)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Chemical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Physical models of vascular systems are being used extensively in our laboratory to study a variety of phenomena related to hemodynamics. Also, computational fluid dynamics calculations are being done with a software package called FIDAP that will simulate momentum, heat, and mass transport in representative arterial systems. During the past year, efforts have been directed to projects in the following categories: (1) a study of intra-arterial drug delivery, using a glass model of the pelvic arteries, in order to optimize the treatment of cervical cancer; (2) a study of the effect of fluid shear on the expression of the gap-junction molecules in endothelial cell membranes called connexons; (3) experimental and numerical analysis of pulsatile flow in branching geometries to simulate fluid forces and arterial-wall transport of macromolecules as they relate to atherosclerosis.

OBJECTIVES: (1) Intra-arterial administration of drug solutions can provide a regional advantage to cancer therapy by yielding a high local concentration of drug in a tumor region while maintaining lower, less toxic systemic levels. Radiation-sensitizing drugs, in combination with radiation, have been used with good success in treating gynecological cancers. To achieve optimum success in treating cervical cancer, the radiosensitizing drug would be delivered only to the tumor-bearing region and would avoid other sensitive tissues around the uterus and rectum. *In vitro* flow models of the cervical artery network allow us to study proper placement of infusion catheters in the uterine artery and to assess different methods of drug delivery that will insure adequate distribution of the drug to the tumor site, while minimizing levels elsewhere.

(2) Vascular endothelial cells are important mediators in numerous biological processes, including regulation of vascular tone, inflammation, and atherogenesis. Cultured endothelial cells have been shown to have unique responses to fluid shear stresses *in vitro*, as they do *in vivo*, e.g., morphological alterations and expression of biological components that affect cell adhesion and transport. Gap junctions allow communication between cells by exchange of cytoplasmic molecules between cells via interconnecting channels made of large proteins that are individually called connexins. This communication is important in control of cell proliferation and differentiation. Although numerous physiologic factors have been shown to regulate gap junctions *in vivo* and *in vitro*, no studies have yet been reported showing a correlation between fluid shear on endothelial cells and changes in gap-junction activity. These factors may be relevant to the genesis of atherosclerosis. A cell-culture apparatus that exposes endothelial cells to controlled fluid shear stresses provides an *in vitro* method of studying the relationship between hemodynamics and atherogenesis. Such an apparatus can also be used to study shear-stress effects on the production of other biological components from the endothelial cells.

(3) Atherosclerosis has a predilection for appearing at specific arterial sites that are associated with low shear or disturbed flow patterns. Flow branching and curvature are geometries that can promote these conditions. The relationship between the flow phenomena and the mass transport of molecules and other blood-borne elements into the arterial wall is essential to our understanding of the etiology of the disease process. We propose to conduct an experimental study, in conjunction with concomitant numerical flow and transport analysis, of a bifurcating flow model that possesses many of the features of flow in real arteries, and to investigate the uptake of various molecular-weight molecules into the "arterial wall." Experimentation and theory can be compared for steady and pulsatile flow conditions.

METHODS EMPLOYED: (1) A flow model of the arterial network of a female human, including branches of the uterine and cervical arteries, was fabricated from glass, using published anatomical illustrations as a guide. The model is connected to a flow system that generates pulsatile-flow waves. The numerous branches of the pelvic-artery model are connected to a collection device that allows individual sampling of the effluent fluid (simulated blood) from these branches. A red dye is injected via a catheter at various locations in the arterial system. The fluid can be sampled from the distal branches to determine the distribution of dye by measuring dye concentration spectrophotometrically or by using a new in-line concentration sensor. This sensor monitors absorbance of the dye in a fluid stream that passes through it, obviating the need for tedious sample collections. The present design of the sensor uses a green-colored LED to emit light, and a photodetector opposed to the LED to measure transmitted light. The fluid containing red dye passes through a glass tube positioned between the LED and the photodetector. The device is interfaced to a computer, which calculates absorbance and records and plots experimental readings. Various injection sites have been tested. Previous experiments have shown that better mixing is achieved with rapid, short bolus injections instead of slow, steady infusions. A simple pulse-infusion device is being designed that will use an electrical solenoid to push-pull a syringe to deliver short boluses of injectate through a series of one-way valves. Flow visualization studies are recorded on videotape to maintain a visual record of the infusion experiments.

(2) A cell-culture chamber was fabricated as a parallel-plate design. Two stainless-steel plates are separated by a thin Teflon sheet to produce a gap for flow of culture media through the chamber. Two windows are cut from the center portion of the rectangular plates, and two glass microscope slides are secured in the window openings to allow observation of the cells, which are seeded on the lumenal surface of the bottom window. The flow chamber is connected by silicone tubing to glass reservoirs, one of which is elevated to provide an inlet head for the steady flow of fluid. The other reservoir serves as the effluent collection tank for eventual recirculation of the fluid (medium) via a peristaltic pump to the head tank. The entire system, including an Olympus phase-contrast microscope, is housed in an incubation chamber made of Styrofoam board and maintained at a constant temperature of 37°C by a regulated hot-air blower. Fluid shear stresses are regulated by adjusting the head-tank level, adjusting flow resistance with pinch clamps, or changing the gap spacing of the parallel-plate chamber. Bovine endothelial cells are grown to confluence in monolayer culture and are subjected to shear stresses from 1 dyn/cm^2 to 15 dyn/cm^2 for durations of 2 to 16 hours. The endothelial cells are then fixed on the removable glass cover-slip windows and sent for assay for activity of m-RNA for the connexin-43 gap-junction protein.

(3) An experimental system is being designed that will consist of a two-dimensional bifurcating flow channel. Portions of the wall of the flow channel will be constructed of a layer of ultrafiltration membrane of known molecular cutoff and an adjacent

layer of gel material to act as the wall media. Fluid will flow through the channel either in a steady stream or in pulsatile bursts, and will contain various concentrations of test molecules of various molecular weights. The topographical distribution of these molecules will be measured at different times and locations in the wall. If possible, fluid side concentrations will be determined at various wall locations as a means of estimating concentration polarization effects locally. FIDAP will be used to simulate these flow and transport conditions in an attempt to provide a unified theory that will explain and corroborate the experimental data.

MAJOR FINDINGS: (1) Our results indicate that streaming of infused solution can occur in pelvic arteries when slow, steady infusion rates (2 ml/in) are used. The ultimate distribution of infused material into distal arteries is a strong function of catheter position. Reinsertion of the catheter into the model to a nominally identical position in the uterine artery, with a degree of accuracy comparable to that expected in a clinical situation, showed a significantly different pattern of distribution from insertion to insertion. Positioning the catheter in the hypogastric artery, a common location for prior clinical studies, showed that a significant amount of streaming can direct the injectate into the arteries of the rectum and bladder, where it can present undesirable toxic effects, and can bypass the uterine artery, which feeds the desired target tissue. Rapid bolus injections from a pulsed-infusion pump greatly improve the distal distribution of injectate. The in-line absorbance monitor (ILAM) has been fabricated and calibrated. A dedicated PC-controlled data acquisition system has been implemented. The ILAM readings correlate well with the usual method of spectrophotometric readings of sampled collections. The new ILAM will be used to test the new solenoid/syringe bolus-injection system.

(2) The parallel-plate, fluid shear cell-culture system is being used to test shear effects on cultured bovine arterial endothelial cells (BEACs). Several studies have been done at 5 dynes/cm^2 and at 15 dynes/cm^2. At 15 dynes/cm^2, reorientation of the cells occurred by 17 or 18 hours of shear-stress exposure. Samples of the endothelial cells have been fixed and analyzed for connexon protein m-RNA. We found up-regulation of m-RNA for connexon-43 at shears of 5 dyn/cm^2 for 5 hours. This activity appeared to drop at 24 hours for 5 dyn/cm^2. This up-regulation effect seemed greater at 5 dyn/cm^2 than at 15 dyn/cm^2.

(3) Dialysis and ultrafiltration membranes are being tested in conjunction with agarose and polyacrylamide gels to simulate the diffusive and hydraulic flux properties of artery walls at the intima and media. A bifurcating flow system with a semipermeable wall is being designed to study the transport of several molecular-weight molecules in steady and pulsatile flow. FIDAP is being programmed to simulate the flow and transport through the

wall of the bifurcating system, and these simulations will be correlated with experiments.

SIGNIFICANCE: (1) To achieve maximum therapeutic benefit with minimum toxicity to the patient, proper delivery of drug solution to the desired tumor-bearing region must be achieved. *In vitro* flow models are very useful in assessing delivery methodologies (e.g., catheter placement, catheter design, and injection procedures). Catheter placement is critical for targeting the desired tissue region for drug delivery. Rapid bolus injections can enhance mixing.

(2) The vascular system is complex. Vascular cell function has been shown to be modulated by fluid shear forces. Endothelial surface molecules can be regulated by local hemodynamics. Understanding the influence of fluid mechanical forces on cell surface-molecule function can lead to a better understanding of the homeostatic mechanisms and of the etiology of some diseases (e.g., atherosclerosis).

(3) Atherosclerosis is associated with the accumulation of lipoprotein deposits in the arterial wall at sites of low shear stress and disturbed flow patterns. An understanding of the transport mechanisms that lead to this accumulation will aid our understanding of the etiology of the disease process. *In vitro* models of flow and transport are useful tools in this study, as are mathematical models, to corroborate experiments and provide a unified theory to explain the uptake of lipoproteins.

PUBLICATIONS: Peacock J, Hankins S, Jones T, Lutz R. Flow instabilities induced by coronary artery stents: assessment with an *in vitro* pulse duplicator. J Biomechanics 1995;28:17-26.

Lutz R, Epstein A, Cook J, Dedrick R. An in vitro flow model to study streaming during pelvic intra-arterial drug infusions. Gynecol Oncol 1995 (accepted).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10327-07 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mass Mapping of Macromolecular Assemblies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Richard D. Leapman, Ph.D. Physical Scientist OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
LN, NINDS (S. B. Andrews, P. E. Gallant, T. S. Reese); LSBR, NIAMS (A. C. Steven)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Neurofilaments (NFs), a neuron-specific subtype of intermediate filament (IF), are an essential structural component of the axon. Although the sequence and secondary structure of NF subunits are established, the higher-level organization of subunits into ~10-nm-diameter filaments remains unknown, in part due to compositional heterogeneity and difficulties in obtaining native preparations. Squid NFs contain low- (L-) and high- (H-) molecular-weight subunits (M_r = 60 kDa and M_r = 120 kDa, respectively). The N-terminal and ~50-nm-long alpha-helical rod domains of the L- and H-subunits are identical, but the H-subunit has an additional C-terminal tail containing 63 potential phosphorylation sites. These highly charged phosphorylated tails are believed to stabilize the NF cytoskeleton. A novel combination of scanning transmission electron microscopy (STEM) and parallel electron energy-loss spectroscopy (PEELS) has been used to show that native NFs isolated from squid giant axon are essentially fully phosphorylated and contain only 8±1 coiled-coil dimers per cross section.

OBJECTIVES: To determine the higher-level organization and phosphorylation state of neurofilaments isolated from the giant axon of squid.

METHODS EMPLOYED: Native squid NFs from squid giant axon were purified by mildly dissociating squid axoplasm in potassium aspartate buffer and passing the dissociated axoplasm through a discontinuous sucrose density gradient. Protein was injected into a drop of buffer placed on the EM grid to avoid possible denaturation. Rapid freezing was accomplished by adsorbing the NFs onto thin ~3 to 4-nm carbon films supported over a thicker lacy carbon film; the grids were washed in volatile ammonium acetate buffer, blotted and immediately plunged into liquid ethane at -160°C. Grids were cryotransferred into a VG Microscopes HB501 STEM and were slowly freeze-dried at -110°C before recooling to -160°C. Digital dark-field images were recorded at an electron dose of ~1000 electrons per square nanometer to avoid mass loss.

MAJOR FINDINGS: Quantitation of the STEM images using the TMV mass calibration gave a value of 22.7±0.8 kDa per nanometer for the NF mass-per-unit-length. From the known subunit masses and the axial repeat distance of ~50 nm, we can deduce that native squid NFs are composed of 8±1 coiled-coil dimers per cross section. If the L:H subunit ratio is 3:1, then each NF contains twelve L-chains and four H-chains per cross section. Our results differ from STEM mass measurements on other IFs which indicate 16 coiled-coil dimers per cross section.

SIGNIFICANCE: We have shown for the first time that it is feasible to measure the mass and phosphorylation state of individual protein molecules with a sensitivity of around 5 phosphorus atoms. The STEM/PEELS measurement of phosphorylation may become a generally useful technique for studying regulatory proteins contained in large macromolecular assemblies.

PROPOSED COURSE: STEM mass-mapping and measurement of phosphorylation level will be applied to other proteins.

PUBLICATIONS: Leapman RD, Sun S, Shi S, Gallant PE, Reese TS, Andrews SB. Biological electron energy loss spectroscopy and mapping in the field-emission STEM. In: Jouffrey B, Colliex C, eds. Proceedings of the International Congress on Electron Microscopy. Les Ulis, France: Les Editions de Physiques, 1994;3B:801-4.

Leapman RD, Andrews SB. Analyzing macromolecular assemblies and cellular organelles by electron energy loss spectroscopy in the STEM. In: Proceedings of the 23rd FEBS meeting. 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10331-06 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microanalysis of Rapidly Frozen Tissue in the Field-Emission STEM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR
Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR
Carol Swyt, M.S.	Physical Scientist	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

LN, NINDS (S. B. Andrews, T. S. Reese); LMMB, NIAAA (R. Veech);
LPD, NIAID (T. E. Nash); LDN, NICHD (D. V. Agoston)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.8	PROFESSIONAL: 0.8	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Scanning transmission electron microscopy (STEM), electron energy-loss spectroscopy (EELS), and energy-dispersive x-ray spectroscopy (EDXS) have been developed and applied to the measurement of calcium and other ions in neurons. Improvements in cryosectioning technique have been made, including a new accurate method for measuring the thickness of hydrated cryosections at low electron dose. These methods have been applied to the microanalysis of mouse hippocampus. Organotypic cultures of mouse hippocampus grown under well-defined physiological conditions (resting state or electrical stimulation) were rapidly frozen, cryosectioned to a thickness of 100 nanometers, and analyzed by x-ray microanalysis in a high-resolution scanning transmission electron microscope. Ionic concentrations were measured in dendrites of the CA1 region of the hippocampus. Preliminary results indicate that calcium concentrations are elevated in membrane-bound cisterns of dendritic endoplasmic reticulum in stimulated cells, whereas calcium is present at lower levels in the controls.

OBJECTIVES: To determine subcellular ion distributions in rapidly frozen tissue by analytical electron microscopy. To investigate the role of calcium in synaptic activity in hippocampal neurons.

METHODS EMPLOYED: Cryosections of rapidly frozen hippocampus were transferred into a high-resolution cryo-STEM. Digital dark-field images were acquired from selected areas of the freeze-dried cyosections to characterize the structures of interest. X-ray spectra were recorded by means of an ultrathin-window energy-dispersive spectrometer, and were quantified using the DTSA software running on a Macintosh II computer.

MAJOR FINDINGS: Cryosection thickness is an important parameter in optimizing elemental microanalysis because of overlapping structure and difficulty in interpreting the resulting data, and also because plural inelastic scattering limits the sensitivity for EELS. Compression has been found to result in an increased thickness by a factor of about two, and hydrated sections nominally cut to 100 nanometers are measured to have a thickness of approximately 200 nanometers. Shrinkage during dehydration generally results in a 50% further increase in dry mass, while an additional contribution comes from the support film. Preliminary results indicate that calcium concentrations are elevated in the endoplasmic reticulum of hippocampal dendrites when the cultured neurons are electrically stimulated, whereas calcium is present at lower levels under control conditions.

SIGNIFICANCE: Calcium, which is an important intracellular second messenger, is believed to modulate neuronal activity. Direct evidence for the accumulation of calcium in storage organelles of the endoplasmic reticulum of dendrites in hippocampus provides important information about the mechanisms for uptake and release of calcium in the brain. It has been suggested that this process may be involved in learning and memory.

PROPOSED COURSE: Cryo-preparation and microanalytical techniques will be developed further, and will be used to measure calcium concentrations in neurons and in other tissue (or cells) under well-defined physiological conditions.

PUBLICATIONS: Shi SL, Sun SQ, Nash TE, Leapman RD. Characterization of cryosectioned cell suspensions by STEM. In: Proceedings of the 52nd annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1994;136-7.

Shi S, Sun S, Andrews SB, Leapman RD. Thickness measurement of hydrated and dehydrated cryosections by EELS. Microsc Res Tech (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10337-06 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

High Single-Rate NIM module

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen Markowitz, M.E.E.	Electrical Engineer	ACES, BEIP, NCRR
Burton Chidakel, B.S.	Instrument Maker	BEIP, NCRR

COOPERATING UNITS *(if any)*

NM, CC (M. V. Green)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section, CCIS

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

Total Staff Years: 0.2	Professional: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

X-(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

_(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Nuclear Medicine Department of the Clinical Center is developing a multipurpose gamma-ray imaging system for assessing organ function in small animals and in human subjects. For this system, a Nuclear Instrumentation Module (NIM) was designed and constructed to improve count-rate performance without a serious loss of spatial resolution. This module included tap passive delay lines with high rise time (RT) to delay time (DT) ratios (RT/DT = 10) and high-bandwidth (800 mhz), high-slew rate (1200 V/us) amplifiers.

OBJECTIVES: The relatively slow decay time of scintillator crystals limits the count-rate capability of event pulses. A technique of pulse shortening was required to return event pulses to baseline quickly, to prevent system stagnation.

METHODS EMPLOYED: The unit is a single-width NIM module with an input and ouput impedance of 50 ohms. The preamplifier output is input to the unit via a BNC connector on the front panel. The unit involves taking the preamplifier output and multiplying it by a fraction f. The fraction will be variable from 10% to 50% by front-panel control. The attenuated signal is then inverted and delayed. The delay is selected from internal jumpers, and is variable from 50 nanoseconds to 350 nanseconds. The sum of the attenuated inverted signal and the preamplifier input is delayed and then ouput from the module via a BNC connector on the front panel. The second delay is operator-selected from internal jumpers, and will be in the 200-nanosecond range.

SIGNIFICANCE: The unit significantly improves count-rate performance, allowing higher-rate processing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10339-06 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Support of Laboratory of Diagnostic Radiology Research

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ronald L. Levin, Sc.D. Biomedical Engineer MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
MRIPS, LDRR, OIR, OD (J. A. Frank, J. L. Ostuni, H. Surova, C. Vinod); DSB, DCRT (M. A. Douglas)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.9	0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In 1989 Congress asked the NIH to expand its research to include aspects of radiology and imaging. To this end, a Laboratory of Diagnostic Radiology Research (LDRR) was established. This program provides radiologists and others from the American radiological community with training in research methodology and techniques. Part of this training includes performing research with NIH staff scientists and clinicians, using the latest techniques for whole-body magnetic resonance imaging and for visualization and multidimensional analysis of multimodality medical images. Additional NMR instrumentation and image-processing hardware and software have been acquired. The BEIP is providing logistical and scientific expertise in support of this program.

OBJECTIVES: (1) To facilitate magnetic resonance research in the fields of imaging, spectroscopy, angiography, perfusion, and diffusion. (2) To facilitate the exchange of medically related images by permitting images from multiple sources to be accessed from a single archive and in a single file format, and to facilitate the manipulation and comparison of medically related images through the use of a specially designed biomedical workstation.

PROPOSED COURSE: (1) A Signa 1.5-T whole-body magnetic resonance unit being used by this program is continually upgraded so that it remains at the state of the art. Recent upgrades include the addition of specialized gradient and RF coils to facilitate functional and breast imaging.

(2) A multidimensional radiological image-processing system (MRIPS) has been deployed, the primary use of which will be the visualization and analysis of multimodality medical images. The system consists of a combination of computer software and hardware suitable for the development of new image-visualization and image-analysis tools, as well as for the use of existing image-processing software packages.

The MRIPS consists of the following hardware and software: (1) a network of image-visualization and image-analysis workstations, supported by four centrally located data servers containing over 300 Gigabytes of data; (2) the UNIX operating system; (3) an X11/MOTIF graphic user interface; (4) software tools for the development of new image-visualization and image-analysis programs; (5) an image-analysis application; and (6) network and system software compatible with existing NIH computer networks and image-processing programs.

The MRIPS will be used by trainees associated with the LDRR and by other NIH scientists for the analysis of medical images obtained by computerized tomography (CT), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), positron-emission tomography (PET), etc. Many of these studies will involve determination of the relationships among anatomical and physiological image data that have been obtained from various body parts. Of particular importance in this regard is the efficient and accurate spatial registration of MRIPS-collected data.

METHODS EMPLOYED: This system, the Multimodality Radiology Image Processing System (MRIPS), consists of the following components:

(1) AFS (Andrew File System)-based data/computation servers consisting of four DECstation 5000 CPUs and three SUN superSPARCstation CPUs with over 300 Gbytes of magnetic disk space; one SUN superSPARCstation 2 AFS/NFS gateway; one SPARCstation AFS/AFP gateway; one HP 700/930 AFS-client database and Kerberos server; associated networking and communication subsystems; and other miscellaneous peripherals.

(2) Numerous AFS-based and NFS-based workstations consisting of SPARCstations and superSPARCstations, MIPS-based and ALPHA-based DECstation, HP Series 700 machines, and SGI workstations.

(3) Special dataset transfer, format conversion, and data management routines, including Kerberos-based security mechanisms and a dataset registry.

(4) The MEDx image processing software package, consisting of special medically related image-processing enhancements to Sterling, Virginia-based BDS Systems' commercially available nonmedical Xcalibur™ image-processing package.

NIH currently has licenses to use Xcalibur™ on over 100 workstations, as well as on the data/computation servers. There is a large demand from members of the intramural NIH community for additional licenses to use MEDx on existing workstations, in addition to the need to acquire additional workstations for those individuals/laboratories needing to do medical image processing, but lacking appropriate UNIX-based workstations. There is also a demand for enhancements to be made to MRIPS, in addition to long-term support and training.

RECENT ACCOMPLISHMENTS: (1) Deployment of seven AFS-based file servers with over 300 Gigabytes of storage, plus ancillary hardware and software; (2) deployment of three gateway machines that facilitate the automatic movement of data from all of NIH's digital CT and MR scanners into the MRIPS Data Registry (this corresponds to the collection of over 4 GIGAbytes of data per day); (3) deployment of the MEDx image visualization and analysis package on five different platforms (e.g., Sun SPARC, DEC MIPS, DEC Alpha, HP PA_RISC, and SGI MIPS). Also, the deployment of the ANALYZE, IDL, and PV-WAVE packages on five different platforms (e.g., Sun SPARC, DEC MIPS, DEC Alpha, HP PA_RISC, and SGI MIPS); (4) registration of over one hundred and fifty authorized MRIPS users, and training of over one hundred NIH personnel in the use of MRIPS/MEDx.

FUTURE PLANS: (1) To continue to facilitate the exchange of medically related images by permitting images from multiple sources (e.g., CT, MR, PET, etc.) to be accessed from a single archive, independent of the original format of the data; (2) to continue to facilitate the manipulation and comparison of medically related images through the use of a specially designed image visualization and analysis package, e.g., MEDx; (3) to enhance the current MEDx package by adding features and functions requested by the NIH ICDs (e.g., debuggers, enhanced 3-D manipulation tools, etc.); and (4) to explore the possibility of extending the current package to nonradiological images, such as electron micrographs and histopathological images.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10343-06 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polynomial Spline Signal-Processing Techniques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR
Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR
Chulhee Lee, Ph.D.	Visiting Fellow	OD, BEIP, NCRR
Philippe Thévenaz, Ph.D.	Visiting Fellow	OD, BEIP, NCRR
Michael Vrhel, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects   _(b) Human tissues   X _(c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Conventional digital signal-processing techniques consider signals that are represented by a set of uniformly spaced sample values. Although most processing algorithms are derived within a purely discrete framework, there are a variety of biomedical problems (e.g., detection of anatomical structures in images, registration) that would be better formulated by considering a signal as a continuous real-valued function defined over some domain. This study is concerned with the development of such new processing techniques that represent signals and images by continuous polynomial spline functions. What makes this type of approach feasible is the availability of fast algorithms for spline interpolation and approximation that we developed previously. The spline formulation provides a sound mathematical foundation for this type of problem, and typically results in algorithms that outperform the best conventional approaches. We have shown that these techniques are especially appropriate for performing geometrical transformations of images (scaling, rotation, affine transformations). Our new high-quality spline-based methods have been found to be extremely useful for the reslicing of volumetric data (PET, MRI, and fMRI). They have also resulted in the design of fast algorithms for the wavelet transform.

OBJECTIVE: To develop new processing techniques using continuous polynomial spline representations of signals. These techniques can be useful for resolution conversions (zooming and shrinking), geometrical transformations, data compression, multiscale signal analyses, edge detection, or signal differentiation.

METHODS EMPLOYED: The whole approach is based on the premise that any polynomial spline function can be represented as a weighted sum of shifted B-spline basis functions. A signal is therefore entirely characterized by the discrete sequence of its B-spline coefficients, which can be computed efficiently by recursive digital filtering. Using such continuous/discrete signal characterizations, we can design a variety of continuous signal processing operators (e.g. affine transformation, differentiation). The principle is to apply the operator to our continuous input model, and then compute the least squares approximation of the result in a given spline output space. Thanks to our more recent theoretical results, we also have a full control of the approximation error.

MAJOR FINDINGS: During this year, we have primarily developed spline-based procedures for the geometrical transformation of images, including translation, scaling, and rotation. Using high-order spline models, we have been able to obtain results that are better than those obtained using any traditional rescaling method. We have also improved the speed of these transformations by introducing new factorizations of a rotation matrix. In this way, we have an entirely separable formulation. In other words, we can obtain higher-quality results with less computation.

SIGNIFICANCE: To provide NIH scientists with new tools for the processing of biomedical signals. To increase the efficiency of resolution conversion and display algorithms on image processing workstations.

PROPOSED COURSE: We will continue our investigation of spline representations and explore the potential of new approximation methods suggested by our very recent theoretical developments. We will also apply our new factorization methodology to speed up a spline-based multiscale registration algorithm which is currently applied to the alignment of 3-D functional images of the brain (PET, fMRI).

PUBLICATIONS: Aldroubi A, Unser M, Eden M. Discrete spline filters for multiresolutions and wavelets of l_2. SIAM J Math Anal 1994;25(5):1412-32.

Unser M, Aldroubi A. Fast wavelet signal analyses: design and algorithms. In: Proceedings of the Workshop on Time-Frequency, Wavelets, and Multiresolutions. Lyon, France: 1994;9.1-9.10.

Unser M, Aldroubi A. Fast algorithms for running wavelet analyses. Proc SPIE (Wavelet Applications in Signal and Image Processing II) 1994;2303:308-19.

Unser M, Neimark MA, Lee C. Affine transformations of images: a least squares formulation. In: Proceedings of the IEEE International Conference on Image Processing. Austin, TX: IEEE, 1994;3:558-61.

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Vrhel MJ, Lee C, Unser M. Fast continuous wavelet transform. In: Proceedings of the IEEE International Conference on Acoustics, Speech and Signal Processing. Detroit, MI: IEEE, 1995;2:1165-8.

Unser M, Aldroubi A. Optimal sampling of non-bandlimited signals with applications to image processing. In: Proceedings of the IS&T's 48th Annual Conference. Washington, DC: 1995;370-5.

Unser M. A general Hilbert space framework for the discretization of continuous signal-processing operators. Proc SPIE (Wavelet Applications in Signal and Image Processing III) 1995 (in press).

Unser M, Thévenaz P, Yaroslavsky L. Convolution-based interpolation for fast, high-quality rotation of images. IEEE Trans Image Process 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10353-05 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Drug Transport in Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul F. Morrison, Ph.D.	Physical Scientist	CHES, BEIP, NCRR
Peter J. Basser, Ph.D.	Mechanical Engineer	MES, BEIP, NCRR
Robert L. Dedrick, Ph.D.	Chief	CHES, BEIP, NCRR

COOPERATING UNITS *(if any)*
SNB, NINDS (D. Laske, D. Lieberman, M. Corthesy, E. Oldfield);
CC (J. Reynolds)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Theoretical and experimental transport models of high-flow microinfusion into brain tissue have indicated the feasibility of delivering macromolecules over cm distances in either grey or white matter. To address remaining issues of infusion control and monitoring, a study was undertaken in monkeys with 111-In-DTPA-transferrin to determine: (a) penetration distances and volumes achievable with long-term white matter infusions using implantable programmable pumps; (b) long-term clearance rates of the protein from the infused region; (c) the magnitude of the effect of microinfusion on blood perfusion rates to brain; and (d) whether SPECT could be used to assess these factors noninvasively during the course of infusion. Drug was infused at an average flow rate of 1.9µl/min for 87 hrs, and planar and SPECT images of both the infused protein and regional perfusion marker HMPAO were obtained on days 1,3,4,8, and 11 following beginning of infusion; autoradiography was obtained from another monkey sacrificed 9 hrs post-infusion. An intensity threshold of 39% that was applied to the D-V SPECT image yielded a width matched to the D-V white matter spread measured directly on autoradiograms. A-P, M-L, and D-V spreads at end of infusion were 2.7 cm, 2.2 cm, and 2.9 cm, respectively. Infusion volumes over 3 monkeys ranged from 4.9 ml to 6.7 ml. Post-infusion tissue clearance determined from absorption-corrected planar images was 0.118±0.033 µl/min/ml, suggestive of clearance by extrachoroidal fluid production and flow. Local perfusion reduction was small (on the order of 3.4%).

RELEVANT BEIP PROJECTS: 84-125, Drug Transport in Brain.

OBJECTIVES: To understand the fundamental processes involved in the transport of small- and macromolecular-sized molecules in the white and grey matter of the brain. To use this information to predict the distribution of these molecules following various administration protocols, and to assess the role of transport in determining therapeutic or diagnostic outcome.

METHODS EMPLOYED: Experimentally and in collaboration with Surgical Neurology and the Clinical Center, anesthetized monkeys were implanted with Medtronic programmable pumps and catheters. Catheter slits were centered in the semiovale centrum of one hemisphere. Pumps were filled with a 5nM 10ml solution containing 100µCi of 111-In-DTPA-apotransferrin. 3 days after emplacement, infusion was begun at 0.5µl/min and then increased over 2 hrs to 2µl/min; infusion was complete at 87 hrs. At days 1, 3, 4, 8, and 11 after initiation of infusion, planar and SPECT scans were obtained with TRIAD88 3-gamma camera instrumentation. 10mCi of 99m-Tc-hexamethylpropyleneamine oxime (HMPAO) was administered i.v. at the beginning of each scan. Forty 111-In-transferrin projections, each consisting of a 64x64 matrix, were collected over a period of 30 minutes. Data were reconstructed by back-projection with standard commercial software using the Butterworth filter with no attenuation or scattering corrections. Reconstructed slices were 1 pixel thick, with a scaling factor of 2.22 mm/pixel. In addition, planar left and right lateral images (256x256 matrices with 1.11 mm/pixel) were each obtained from a single gamma camera with a collection time of 5 mins. Subsequent data analysis was performed with the NIH program Image v1.57 (Wayne Rasband). For autoradiography, one additional monkey was infused with ^{111}In-DTPA-transferrin solution containing 1.715 mCi at start of infusion; this dose was chosen to optimize film sensitivity at the time of sacrifice. Infusate preparation, catheter placement, infusion rate, and total volume were as above. At 10 hrs post-infusion, SPECT and planar images were obtained, followed 3 hrs later by sacrifice and removal of the brain. Sections 20 µ thick were cut from the brain. Autoradiographic standards were prepared by mixing a 1:9 (infusate:stock) gel solution, followed by five serial 1:1 dilutions. These gels were frozen, cut into slices 20 µ thick, and affixed to glass slides. Brain sections and standards were exposed to HP film for 5 days and then developed. Film images were digitized using a CCD camera with video capture to the NIH program Image v1.57. Images were distance-scaled and calibrated to the approximately linear autoradiographic standards. For comparison with theory, recently published transport models were employed that were constructed from differential representations of Darcy flow, diffusion, bulk flow, and continuity applied to a porous model of the brain. Binding, biochemical reaction, and capillary pore bulk flow and permeation were also included as appropriate sink terms.

MAJOR FINDINGS: Autoradiography revealed that the infusion resulted in a frontal white matter volume filled nearly homogeneously, with some lower-concentration spillover into surrounding grey matter. SPECT images are poorly resolved at the infusion boundaries, but an intensity threshold of 39% applied to coronal images was found to reproduce the D-V dimension directly measureable on autoradiograms. Protein spread and distribution volumes were large and on the cm scale, as expected: A-P, M-L, and D-V spreads at the end of infusion were 2.7 cm, 2.2 cm, and 2.9 cm, respectively. Infusion volumes over 3 monkeys ranged from 4.9 ml to 6.7 ml. Protein clearance for a negligibly metabolized compound such as 111-In-transferrin was found to be slow, as determined from the serial decline in total radioactivity in both absorption-corrected lateral planar images and slice-summed SPECT images. Clearance was 0.118±0.33µl/min/ml of tissue, corresponding to a post-infusion clearance half time of 4.1 days, and suggestive of tissue clearance due to extrachoroidal production and flow of CSF. HMPAO scans, contrasting the infused and noninfused hemispheres, revealed only small reductions in local blood perfusion, the average decline being only 3.4%.

SIGNIFICANCE: The large spreads of macromolecules obtained in this study confirm the ability of microinfusion to deliver drugs to a large tissue volume in brain, with relatively flat concentration profile. The lack of greatly reduced perfusion to the tissue following infusion, coupled with histological evidence of only minor local gliosis and no obvious clinical manifestations, provides further indication that this high-flow technique may be safely employed in therapy. The slow clearance of In-DTPA-transferrin is indicative of the long retention times that can be obtained for slowly metabolized proteins, and of the need to account for proper post-infusion clearance when estimating tissue response. Finally, SPECT has been demonstrated as being capable of monitoring the spread of agents able to undergo labelling with a gamma-emitting radionuclide.

PROPOSED COURSE: A more complete characterization of the infusion operating conditions will be undertaken to assure maximum retention of infused agent in brain tissue. Catheter diameters and depths of insertion are frequently altered in different applications, yet no description is available that relates these parameters to flow rate, tissue permeability and elasticity, and the probability of leakback. In addition, pressure measurements and autoradiography following small-volume infusions will be conducted to allow determination of isotropic and anisotropic hydraulic conductivities in grey and white matter.

PUBLICATION: Lieberman DM, Laske DW, Morrison PF, Bankiewicz KS, Oldfield EH. Bulk flow enhances the distribution of large molecules in gray matter during direct interstitial infusion. J Surg Neurol 1995;82:1021-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10358-05 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Heat Capacity Effects in Lipids During Unilamellar/Multilamellar Phase Changes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Courtney P. Mudd, Ph.D.	Biomedical Engineer	ACES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electronics Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LPB, NIAMS (N. Gershfeld); IR CH, NHLBI (R. Berger)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent studies of the properties of phospholipid dispersions in water indicate that a higher-order phase transition occurs that involves a spontaneous transformation from a unilamellar liquid-crystal state to a suspension of large, unilamellar vesicles when the ambient temperature increases. It has been suggested that the unilamellar vesicles that form are a critical state. The thermodynamic properties of this transformation have been inferred primarily from the properties of air-water surface films in equilibrium with the dispersed phospholipid phase. A more direct and conceptually simpler analysis of the thermodynamic properties of this higher-order transition may be obtained by measuring the temperature dependence of the heat capacity of the lipid dispersions. Since transformations of this type are believed to be intimately involved in the assembly of cell membranes, we developed an extremely sensitive differential heat-conduction calorimeter for measuring heat capacities of aqueous-membrane lipid dispersions. Using this instrument, we have measured a small heat-capacity change during the phase transition at the critical temperature. We have begun work on two other independent measurements to confirm the nature of the transition: (1) a fluorescence system that measures the abrupt increase in surface area of the lipid during the transition from multilamellar to unilamellar, and (2) X-ray diffraction measurements of the intermolecular spacing during the transition.

OBJECTIVES: We now have three primary objectives: (1) to use a newly designed differential (two-cell) batch-type heat-conduction microcalorimeter to step through a set of temperatures in the range of the critical temperature, T*, and to measure changes in the heat capacity of the lipid sample; (2) to mix a fluorescent dye with the lipid in its unfolded state, to cool the lipid back below its phase-transition temperature to its folded state, and then to increase the temperature slowly through the phase-transition temperature while measuring the fluorescence (if the lipid unfolds abruptly, i.e., over a small change in temperature, there should be a sudden increase in the measured fluorescence); and (3) to control the temperature of the sample as we obtain X-ray diffraction patterns related to the intermolecular spacing. Again, an abrupt change in the intermolecular spacing over a small temperature interval will confirm the nature of the phase transition. This scheme will require temperature stability of better than 1 millidegree Celsius in the sample.

SIGNIFICANCE: Equilibrium surface film studies of DMPC (lipid) dispersions have shown that at the critical bilayer temperature, T*, a unilamellar state is formed from liposomes. Since we have now shown that there is a heat-capacity change at this critical temperature, we need verification through other independent measurements of this behavior. If the behavior is confirmed, we will have shown that calorimetry can provide a direct measurement of the work of bilayer adhesion, as well as verification of the value of the critical temperature.

PROPOSED COURSE: The commercial spectrofluorimeter has been purchased, and modifications are under way to improve the temperature control and the scanning capability. This component of the investigation has now been opened as a separate project (q.v.). The X-ray diffraction experiment will require a redesigning of the lipid sample holder in order to prevent the lipid from settling out of the X-ray aperture during the experiment. The location of the heaters close to the sample and the placement of the control thermistor directly in the sample enabled the control system to compensate for the energy dissipation caused by the X-ray beam as it passes through the sample, and to keep the sample temperature stable to within ±0.5 millidegrees Centigrade. We are evaluating cell designs that can rotate the sample every 12 hours, and horizontal mounting that will allow the X-ray to pass vertically through the lipid suspension.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10359-05 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Flash Photolysis of Microsomal P450

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen Markowitz, M.E.E.	Electrical Engineer	ACES, BEIP, NCRR
Burton Chidakel, B.S.	Instrument Maker	BEIP, NCRR

COOPERATING UNITS *(if any)*
LMC, NCI (F. K. Friedman)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section, CCIS

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.15	0.1

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A laser flash-photolysis apparatus was developed to measure the kinetics of carbon monoxide (CO) binding to P450 cytochromes in liver microsomes from rats treated with various drugs and carcinogens. Since numerous forms of P450 contribute to the overall reaction, a difference kinetic method was used to distinguish the kinetic behavior of individual P450s. This method entails analysis of the difference between the kinetic profiles in the absence and presence of a specific P450 effector, and successfully yielded kinetic parameters for individual P450s involved in drug and carcinogen metabolism. Specifically, various polycyclic hydrocarbons differentially accelerated CO binding to the P450 1A1 form, which metabolizes these carcinogens in a size- and shape-dependent manner. In addition to rat-liver microsomes, this approach was applied to a single human P450. Human P450 3A4 was evaluated, and different substrates modulated the CO binding kinetics in a substrate-dependent manner.

OBJECTIVES: To develop an instrument and analytical methodologies that use the CO flash-photolysis technique to measure the kinetics of CO binding to P450. To apply this method as a probe of P450 structure and dynamics, employing specific effectors of P450 function, such as anti-P450 antibodies and substrates, to elucidate the kinetic behavior of individual P450s. The general problem of attributing a rate constant to a specific P450 in the presence of multiple microsomal P450s was overcome by employing a difference kinetic method. This analysis is based on a kinetic profile consisting of the difference between the kinetic curves obtained in the presence and absence of the P450 effector.

METHODS EMPLOYED: A dye-laser flash-photolysis apparatus was designed and constructed to measure CO binding kinetics. The sample is pulsed with a laser flash at 530 nm to disrupt the photolabile bond between CO and the heme iron of the P450. Transmitted light at 450 nm is detected by the photomultiplier. Several optical filters and lenses select the wavelength and focus the light. The photomultiplier voltage output is amplified, filtered, input into an analog-to-digital converter, and stored on the IBM AT. The amplifier with offset was designed to limit A/D quantification noise. The hardware filter was used to limit aliasing effects.

Data from repetitive experiments were averaged to improve the signal-to-noise ratio (S/N). Further S/N enhancement was obtained by a moving point average and fast Fourier-transform techniques.

The CO recombinations were carried out under pseudo-first-order conditions in excess (greater than tenfold) CO. Experimental data were processed and analyzed with RS/1 software using least squares fitting to multiexponential models.

MAJOR FINDINGS: The binding of CO to P450 was monitored by the absorbance change at 450 nm. This approach provided detailed information on the protein structure and dynamics in the heme region for several hemeproteins, but has been less extensively applied to P450.

Microsomes contain a variety of P450s whose individual concentrations are unknown. The capability of resolving these P450s is primarily determined by the sensitivity and noise of the instrumentation, which determine the minimum number of exponentials that fit the data. We circumvented the major problem of attempting to fit a minimum number of exponentials to a larger number of P450s by evaluating the difference between kinetic curves in the presence and absence of effector. This cancels the contributions from the majority of reactive P450s that do not interact with effector, and yields data that derive solely from the effector-specific P450.

The substrate benzphetamine accelerated CO binding to the P450 2B1 form, which metabolizes this substrate, and thus enlarges the CO ligand access channel. We also assessed the effect of a series of polycyclic aromatic hydrocarbons on the CO binding kinetics of the P450 1A1 form, which metabolizes these carcinogens. While all of these compounds accelerated binding, the extent varied according to size and shape. The smaller compounds had a greater effect than the larger compounds. In addition various substrates for human P450 3A4 influence the CO binding rate differentially: individual substrates accelerated, inhibited, or had no effect on the kinetics. The results were explained by a dual mechanism. Substrate can enhance binding by conformational changes on P450, and can sterically inhibit binding.

SIGNIFICANCE: The kinetic data will help to provide a more comprehensive view of the P450-substrate-CO system. From the perspective of P450 dynamics, the kinetics of CO recombination is a powerful probe for the accessibility of small molecules to the heme in the protein interior. The parameters obtained by this approach include the rates at which CO diffuses from bulk solvent into the protein interior. They also yield the relative amplitudes of kinetically distinguishable pathways toward the final P450-CO complex. These parameters reflect both the conformational structure and the dynamics of the P450.

The contribution of a given P450 to the parameter of interest can be gauged in the presence of a microsomal mixture of P450s by the difference method. The results demonstrate that individual P450s can be characterized in their native membrane environment using differential analytical procedures. Previous work in this field yielded information on mixtures of P450s in microsomes of a single P450 in an artificial reconstitution system.

PROPOSED COURSE: Experiments will be performed using liver microsomes from rats treated with various inducing agents that enhance the microsomal content of specific P450s. The effects of known P450 substrates and inhibitors will be assessed. In addition, the integration of human P450s with substrate will be examined.

PUBLICATIONS: Koley AP, Robinson RC, Markowitz A, Friedman FK. Interaction of polycyclic aromatic hydrocarbons and flavones with cytochromes P450 in the endoplasmic reticulum: effect on CO binding kinetics. Biochemistry 1995;34:1942-7.

Koley AP, Buters JT, Robinson RC, Markowitz A, Friedman FK. CO binding kinetics of human cytochrome P450 3A4: specific interaction of substrates with kinectically distinguishable confomers. J Biol Chem 1995;270:5014-18.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10361-05 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rheology of Sickle Erythrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Richard Chadwick, Ph.D. Head, Biomech. Group OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
LCB, NIDDK (A. Schechter); Pennsylvania State University (C. Dong)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects X (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The rheological properties of normal erythrocytes appear to be determined largely by those of the red cell membrane. In sickle-cell disease, the intracellular polymerization of sickle hemoglobin upon deoxygenation leads to a marked increase in intracellular viscosity and elastic stiffness, and also has indirect effects on the cell membrane. In order to estimate the components of abnormal cell rheology due to the polymerization process (and that due to the membrane abnormalities), we have developed a mathematical model of whole-cell deformability in narrow vessels. This model uses hydrodynamic lubrication theory to describe the pulsatile flow in the gap between a cell and the vessel wall. We use published values of normal and sickle cell membrane elastic moduli and of sickle hemoglobin viscous and elastic moduli as a function of oxygen saturation to estimate the cell deformation and relative hydrodynamic resistance as a function of oxygen saturation for sickle erythrocytes. The model should be useful for reconciling the vast and disparate sets of data available on the abnormal properties of sickle cell hemoglobin and sickle erythrocyte membranes--the two main factors that lead to pathology in patients with this disease. We are also developing models to study red cell heterogeneity with regard to intracellular hemoglobin concentration, as well as a large deformation membrane theory that includes bending and stretching.

OBJECTIVES: To increase our understanding of the relative contributions of the red cell membrane and the internal sickle hemoglobin solution to the viscous and elastic components of total cell deformability during passage through the microcirculation, particularly at different oxygen saturation levels.

SIGNIFICANCE: The deformability of erythrocytes is a critical determinant of flow in the microcirculation. Abnormalities of these properties are thought to be the principal cause of the pathophysiology of sickle-cell disease and other hemoglobinopathies. Extensive clinical studies of the effects of the abnormal sickle erythrocytes in patients have been reported. A central challenge in sickle-cell rheology remains, viz., to ascertain the relative importance of the sickle hemoglobin and cell membrane to the overall properties of sickle cells in the microcirculation.

MAJOR FINDINGS: The model indicates that a cell becomes increasingly less deformable when oxygen saturation drops below 40% to 50%, or when polymer fraction rises above 0.4, if the membrane rigidity has a value representative of a normal cell. The flow resistance of the cells increases significantly when oxygen saturation decreases to this critical level. Cell membrane elasticity certainly becomes a major determinant of the overall cell deformability when sickled hemoglobin solution is fully oxygenated. Several factors can contribute to altering the critical value of polymer fraction (or oxygen saturation). For example, increasing the oscillatory frequency of the blood flow tends to shift the critical value of polymer fraction downward. A dependence of membrane rigidity on oxygen saturation can significantly alter this value, as well; in fact, the concept of a critical polymer fraction no longer applies. In other words, small amounts of polymer can significantly affect whole-cell deformability in this case.

PROPOSED COURSE: The model will be extended to take into account the situation in which a cell passes through a very narrow capillary (i.e., the tube dimension is smaller than that of the undeformed particle). In such a case, the bending rigidity of the cell membrane may play an important role. A filtration assay of sickle-cell trait suspensions using nickel mesh will be designed to investigate filtration under oscillatory conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10362-05 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Binding Forces in Receptor-Mediated Cell Adhesion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Alec Eidsath, Ph.D. Staff Fellow MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LTCB, DCE, NCI (C. Saxinger)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
[✓ (b) Human tissues]
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since it is known that the *tat* protein of the HIV will enter the cell *in vitro* and stimulate the production of HIV products, there is almost certainly a receptor site for it on the cell membrane. Segments of this 86 amino acid protein are being synthesized to determine which segment is responsible for binding to the receptor. This phenomenon will be evaluated by quantitatively measuring the binding force between the various segments and several types of human cells grown in tissue culture. We will use a flow cell and image analysis to determine the average shear force needed to detach the cells. The cells will then be attached to the bottom of a 96-well plate, which will be moved automatically from well to well under computer control.

OBJECTIVES: To rank the binding energies of various *tat* fragments and other polypeptides by determining the force necessary to remove bound cells.

METHODS EMPLOYED: We have chosen two methods of applying force to a cell: centrifugal force and shear force. For the experiments using centrifugal force, we will bind cells to polypeptides bound to the bottoms of 96-well plates, and then spin the cells in a medium of a higher specific gravity. Cells that are pulled off are then aspirated, and the number of bound cells is measured using a fluorescence reader. This method is limited by the force that can be put onto the microtitre plates themselves. Fragments that are more tightly bound can then be removed by centrifugal force, and will be analyzed using a flow system, which can generate strong shear forces. Using the flow system together with a microscope enables direct viewing of how the cells deform and eventually separate from the wall. Since the synthesis of the fragments takes place in a 96-well plate, it is very convenient to base the design of the flow cell on the geometry of the plate. We have decided to use a stagnation point flow (a jet of liquid impinging on the surface containing the cells). The shear stress is inversely proportional to the radius, so one would expect to see a clear area near the center of the well where the flow was stronger than the binding energy, and then a resumption of attached cells at a radius where the binding force just overcomes the shear force. A commercial image-analysis system is used to determine this critical radius automatically (the cells will be loaded with a fluorescent dye, and the image will be taken in an epifluorescence setup). The instrument has been designed to fit into a flow hood inside a biohazard room, due to potential dangers from the virus being studied.

SIGNIFICANCE: We think the binding studies are an excellent tool to complement fundamental studies of molecular biology. The direct aim of the project is to determine which amino acids are involved in the binding of the cell to the surface receptor. We intend to compare these results to the three-dimensional configurations produced using molecular dynamics programs. This research will also enable us to improve understanding of the basic immunology of the virus.

PROPOSED COURSE: The final experiments using the centrifugal force field are complete. The stagnation point flow device has been completed, and we are in the process of making it as automated as possible.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10363-05 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Current Dipole Localization Using EEG Data Model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bradley Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR

COOPERATING UNITS *(if any)*

MNB, NINDS (M. Hallett, S. Sato, L. Cohen, D. Ko)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Mechanical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews
(b) Human tissues
X (c) Neither

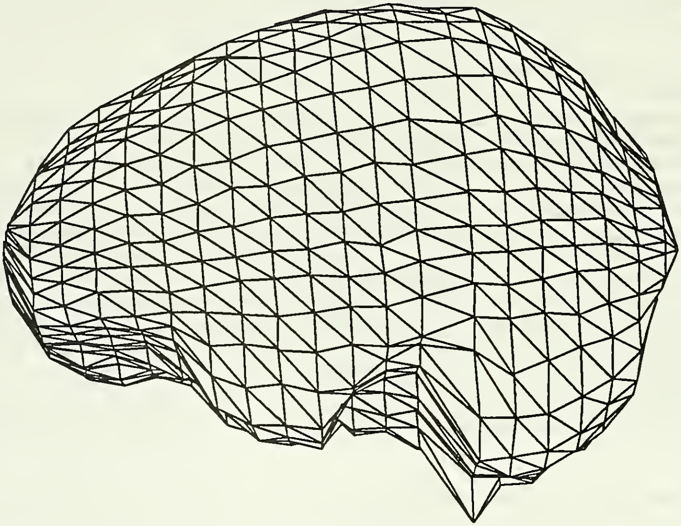
SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A computer program will be developed that can determine the position, orientation, and strength of a current dipole in the brain from electroencephalographic or magnetoencephalographic data. The electrical properties of the head will be modeled using a three-layer model: brain, skull, and scalp. A realistic head shape will be used. The program will be used to localize the focus of epileptic discharges and to analyze evoked potentials. Comparisons will be made between the realistically shaped head model and the three-sphere model. The model will be used to localize the source of interictal spikes in patients who are candidates for epilepsy surgery.

OBJECTIVES: To localize an equivalent dipole source in the brain, using electroencephalographic (EEG) or magnetoencephalographic (MEG) data.

SIGNIFICANCE: The localization of the source of the EEG and MEG is an old problem that has been studied extensively. Until recently, the primary model used to analyze EEG and MEG data was the three-sphere model. It has become clear recently that there are significant errors introduced by modeling the head as a sphere. By using a realistic head geometry, more accurate localizations can be obtained, thus improving our understanding of human cortical organization and enabling surgeons to have more precise knowledge of the areas of the brain responsible for epileptic seizures.

MAJOR FINDINGS: Comparisons between the three-sphere model and the realistically shaped head model indicate that average differences of 2 cm are observed between the two models in the predicted dipole position.



The distribution of 1600 triangles over the brain surface, used to calculate the electrical potential produced by an electric dipole with the realistically shaped head model. The coordinates of the triangles' vertices were obtained by MRI of the subject's head.

PROPOSED COURSE: The first goal will be to verify the model by comparing it to the three-sphere model. Subsequently, the sensitivity of the model to various parameters (e.g., skull conductivity) will be determined. Random noise will be added to the simulations to determine the influence of measurement noise on the results. The model will be used to analyze electroencephalograms (EEGs) from patients who will be undergoing surgery for epilepsy. The calculations will be extended to calculate the magnetoencephalogram (MEG), and studies will be performed to compare the accuracy of MEG and EEG in localizing dipoles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10373-05 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Operation of the *In Vivo* NMR Research Center

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR
Guoying Liu, Ph.D.	Postdoctoral Fellow	NMR, BEIP, NCRR
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR
Alan Olson, M.S.	Electronics Engineer	NMR, BEIP, NCRR
Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR
Denise Sobering, B.A.	Secretary	NMR, BEIP, NCRR
Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR
Peter van Gelderen, Ph.D.	Postdoctoral Fellow	NMR, BEIP, NCRR
Jeannette Black	MRI technologist	NMR, BEIP, NCRR
Rene Hill	MRI technologist	NMR, BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	1.2	2.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of the *In Vivo* NMR Research Center are the advancement of *in vivo* NMR technology; its applications to animal and human physiology; and, ultimately, its application in the clinical setting. The NMR Center's staff collaborate with NIH and other investigators on new methods and original applications of functional magnetic resonance methods, including flow imaging; imaging of capillary circulation; diffusion of water and metabolites in brain and muscle; imaging of oxygen consumption; and imaging and spectroscopy of aerobic and anaerobic glucose metabolism and transport.

Day-to-day operations of the *In Vivo* NMR Research Center include (but are not limited to) scheduling usage of magnets and computers, ordering supplies stocked by the Center for all users, technological support for patient studies, and other activities necessary for a shared resource. In addition, various tasks are directed at the basic operation of any laboratory, such as accounting for usage and funding, submission of necessary documentation, telephone inquiries, and information dissemination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10378-04 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Angioplasty and Atherectomy of Coronary Stenoses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert Bonner, Ph.D.	Physicist	EEES, BEIP, NCRR
Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

MDB, NHLBI (J. Hoeg); CB, NHLBI (S. Epstein); LPS, DCRT (Sinisa Pajevic); Washington Cardiology Center; SCIMED, Inc.; CVIS, Inc.

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects (b) Human tissues X (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In an effort to develop new, more effective forms of angioplasty, we have sought better understanding of the nature of focal coronary stenoses and their acute and chronic responses to intervention. Through the development of the clinical use of intravascular ultrasound (IVUS), we have shown that the composition and stiffness at focal chronic stenoses is remarkably different from adjacent angiographically normal vessel segments, whereas the total mass of atheroma is remarkably similar. The principal lesions currently treated by angioplasty have become rigid due to fibrosis and calcification, which prevent compensatory dilatation observed in more compliant (though heavily diseased) segments. Accordingly, successful interventions disrupt/alter the stiff constraining elements, thus irreversibly making them sufficiently compliant to permit vessel expansion. Successful therapy requires segmental (limited) rupture of the stiff annulus and creation of a large compliant arc. Acoustic transients created by rapid bubble expansion are the principal cause of this disruption in laser angioplasty. Directional atherectomy creates deep focal excisions that can make a small arc (~60°) highly compliant. Rotablators remove luminal calcification, thereby reducing wall stiffness. Following the expansion of "rigid" metallic stents at high pressures, the surrounding tissues generate significant compressive forces that cause an acute and chronic compressive narrowing of treated lesions. The narrowing of these lesions may be the major cause or restenosis.

OBJECTIVES: Current objectives are: (1) to determine the mechanisms of success and failure of angioplasty/atherectomy with devices now being used clinically; (2) to design new devices and methods of angioplasty, based on this new understanding; (3) to develop guidelines for optimal selection of available devices, based on specific lesion characteristics; and (4) to decrease restenosis rate.

METHODS EMPLOYED: We have focused on quantitative morphometry, based both on clinical intravascular ultrasound measurements and pathology of atherectomy specimens and postmortem coronaries, and on the development of animal models of proliferative responses to vascular injury and *in vitro* assessment of device performance. Final evaluation of the angioplasty devices will be based on quantitative intravascular ultrasound examination of clinical lesions before and after therapy, in conjunction with long-term follow-up. Now we are seeking to develop quantitative image-analysis routines for automatically determining the boundaries between lumen/intima and between media/adventitia from intravascular ultrasound images. Such methodology, developed in collaboration with Dr. Michael Unser of BEIP, would be applied to patients in the Molecular Disease Branch, NHLBI, who have severe familial hypercholesterolemia and exhibit extremely rapid childhood progression of disease, in order to perform prospective serial quantitative studies of the mass of atherosclerotic plaque accumulation during the disease's natural progression and during regimens of cholesterol-lowering therapies, which may result in reversal of the disease.

MAJOR FINDINGS: (1) We have shown that the composition and stiffness at focal chronic stenoses is remarkably different from adjacent, angiographically normal vessel segments, whereas the total mass of atheroma is remarkably similar. (2) The principal lesions currently treated by angioplasty have become stiff due to fibrosis and calcification, which prevent compensatory dilatation observed in more compliant (though heavily diseased) segments. (3) Successful intervention relies on the disruption/alteration of stiff constraining elements to make them sufficiently and irreversibly compliant, thus permitting acute and chronically maintained vessel expansion. Successful angioplasty, therefore, works by segmental (limited) rupture of the stiff annulus and the creation of a large compliant arc. (4) Acoustic transients created by rapid bubble expansion during pulsed-laser angioplasty similarly disrupt the stiff vessel segment. Since the volume directly disrupted by the large pressure transients is >100 times the volume vaporized, it is clear that pulsed laser angioplasty is a potent disrupting procedure. Thus, dissection ensues largely from efforts to increase the amount of tissue vaporization, thereby subjecting each plaque element to many (>30) pressure transients. More rapid advancement of the laser angioplasty catheter is proposed to minimize the depth of tissue disruption in order to minimize severe dissection. (5) Directional atherectomy can create a deep focal excision that makes a small arc (~60°) highly compliant, frequently to the point of becoming a pseudoaneurism. (6) Rotablators remove a luminal layer of calcification, subsequently allowing relatively low pressure disruption of the remaining lesion. (7) Following the expansion of "rigid" metallic stents at high pressures, the surrounding tissues generate significant compressive forces that cause significant acute and chronic recoil. (8) These findings suggest that acute and chronic compressive narrowing of treated lesions may be a major contributor to restenosis. (9) In animal models of restenosis, we have demonstrated that transient, moderate (~60°C) thermal elevations associated with thermal angioplasty elicit a profound, dose-dependent proliferative response similar to that seen in severe mechanical injury.

SIGNIFICANCE: Development of more efficacious means of relieving atherosclerotic obstructions in human blood vessels (particularly in the coronary arteries) or of reducing restenosis could have a dramatic effect on the practice of cardiology and vascular surgery. A greater understanding of the composition and structure of atherosclerotic stenoses (as determined by IVUS) and their responses to specific devices could lead to more efficacious selection of devices and refinement of techniques. Dramatic improvements in lowering dissection rate have been observed in preliminary trials of new laser angioplasty methodology. Development of an accurate quantitative method (IVUS) for determining the changes in accumulated plaque mass provides a more quantitative means of evaluating the efficacy of cholesterol-lowering therapies and other medicinal therapies directed at slowing or reversing the development of atherosclerosis.

PROPOSED COURSE: The new focus of this project is to develop an automatic quantitative analysis of intravascular ultrasound images in order to have a reliable method of obtaining values of plaque burden, composition, and resulting vessel-wall morphology, both before and after intervention. Development will be continued of the IVUS database and of the analysis of pre- and post-intervention vessel-wall morphology in patients undergoing angioplasty. In addition, a longitudinal study of rapidly progressive atherosclerosis in young patients with extreme forms of hypercholesterolemia, and of the effects of cholesterol-lowering drugs will be studied, using the quantitative IVUS tools developed.

PUBLICATION: Douek PC, Leon MB, Keren G, Mintz GS, Almagor Y, Bartorelli AL, Potkin BN, Pichard AD, Kent KM, Satler LF, Bonner RF. Failure of compensatory dilation and altered plaque composition at sites of coronary artery stenoses. J Amer Coll Cardiol (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10380-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathophysiology of Syringomyelia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alec Eidsath, Ph.D.	Staff Fellow	MES, BEIP, NCRR
Robert L. Dedrick, Ph.D.	Chief	CHES, BEIP, NCRR
Thomas Talbot, M.S.	Mechanical Engineer	ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*
SNB, NINDS (E. Oldfield, J. Heiss)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects
  (a1) Minors
  (a2) Interviews
(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Syringomyelia is a cavitation of the spinal cord. It most commonly occurs in association with structural abnormalities of the foramen magnum. We plan to assist SNB in the acquisition of pressures in the ventricle, syrinx, and spinal subarachnoid space. The data will be evaluated in connection with anatomic information obtained by cerebrospinal MRI, ultrasonography, and Imatron CT. Correlation of the anatomic and physiologic measurements should help to elucidate the mode of progression of syringomyelia, and may have implications for its treatment.

PHS 6040 (Rev. 5/92)

OBJECTIVES: This study seeks to determine the pathophysiology of the induction and progression of syringomyelia. Establishing the pathophysiology of this disease may have implications for its treatment.

METHODS EMPLOYED: A preoperative series of tests will be conducted in which pressures from the lumbar space and syrinx, along with the EKG trace, will be taken while the subject is awake. Pressures will be monitored during modified Queckenstedt tests (a cuff placed around the neck causing a transient increase in the intracerebral pressure), as well as during such common events as coughing and straining. During surgery, measurements will be obtained at three locations along the spine (the lumbar space, syrinx, and lateral ventricle) in addition to the main arterial and venous pressures. Data from the pressure transducers will be digitized by means of a general-purpose, analog-to-digital board controlled by a "virtual instrument" written in the LabView programming language. LabView was chosen because it gives an easy-to-use graphic interface to the data-collection procedure--a function considered essential to technicians in the operating room. A control study of normal patients was completed this year. A program to synchronize the pressure wave data with the cine MRI results was completed and will be demonstrated soon.

SIGNIFICANCE: The natural history of syringomyelia is one of gradual, progressive deterioration over many years. One objective of surgical treatment is to arrest the disease's progression.

PROPOSED COURSE: Fifteen patients have already been seen, with fifteen additional patients to be included under this protocol. One problem that must be addressed is how to automate the analysis of the large amount of data taken for each patient.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10381-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Patient Electronic Monitoring System, Model II

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horace E. Cascio, BSEE	Electronics Engineer	EEES, BEIP, NCRR
George L. Hemphill, B.A.	Engineering Technician	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
OD, CC (N. Kelly)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.55	0.15	0.40

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Patient Electronic Monitoring Systems (PEMS), which locks the Unit doors and alerts the nursing staff when a patient tries to leave the Nursing Unit, is a welcome aid in the care of patients with Alzheimer's disease or other dementias. A detector unit, placed at each of two exit doors, senses the presence of any patient wearing a wristwatch-size radio transmitter. Upon detection of a patient's transmitter, an embedded computer-based controller locks the exit door, activates an alarm, and displays the patient's name and location on a PC monitor.

The Clinical Center requested that an updated design of the PEMS be installed on the 5E and 3E Nursing Units, and that the obsolete controller on the 6D Nursing Unit be replaced with the newly designed version.

OBJECTIVES: To redesign the PEMS to include state-of-the-art technologies in order to increase system reliability and reduce maintenance. Also, to include all system updates in the 6D PEMS installation.

METHODS EMPLOYED: An improved, printed-circuit patient identifier will be developed using surface-mount integrated circuits and chip resistors and capacitors. The circuit of the identifier will incorporate a crystal-controlled modulator circuit and will be powered by a rechargeable lithium battery. These features will practically eliminate the weekly maintenance routine. The control unit will use an embedded CPU card that allows software program development on a personal computer, in turn eliminating dependency on an obsolete Intel MDS Program Development System. All of the circuitry for the controller will be placed on printed circuit boards in a modular form.

SIGNIFICANCE: The redesign of the PEMS will increase system reliability and reduce the need for technical assistance.

PROPOSED COURSE: Fabrication of the 6D controller is in progress. Upon its completion, the unit will be evaluated and installed on 6D. All of the system updates will be incorporated into the 6D PEMS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10386-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Calculation of Electrical Activity in Cardiac Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bradley J. Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LB, NINDS (J. Clay, V. Kowtha); Dept. of Biomedical Engineering, Duke University (N. Trayanova); Dept. of Physics and Astronomy, Vanderbilt University (J. Wikswo)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

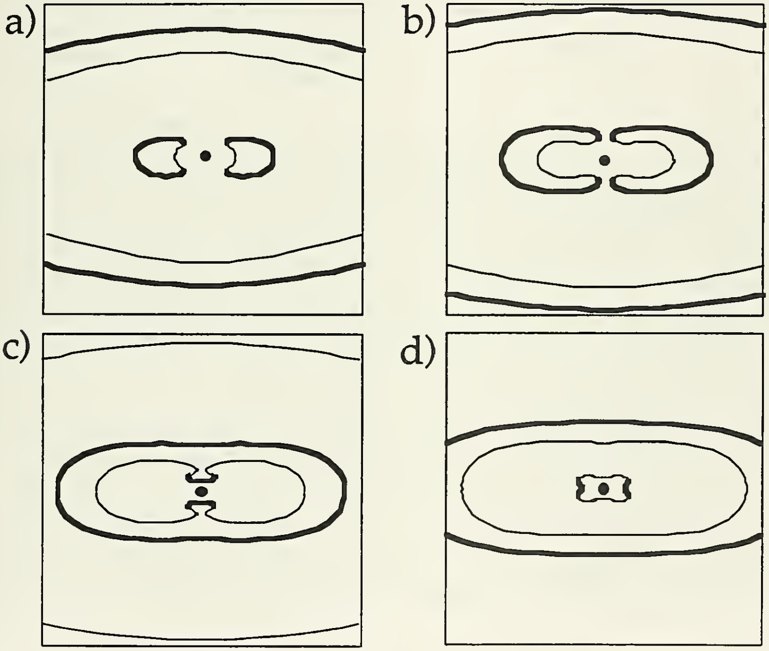
A numerical simulation has been performed of the propagation of an action potential through cardiac tissue. The bidomain model was used to account for the effects of both tissue anisotropy and the interstitial space on current flow. A relaxation technique was used to solve the nonlinear partial differential equations.

The model has been modified to predict propagation in a two- or three-dimensional tissue due to a point source of current. The influence of unequal anisotropy ratios of the intracellular and extracellular conductivities is being investigated. Pairs of stimulus pulses are applied to a two-dimensional sheet of tissue to create rotors and other unusual phenomena, which could prove useful as models of cardiac arrhythmias and fibrillation. The interaction of cardiac tissue with a uniform electric field was also examined. Analytical solutions to the bidomain equations using perturbation theory methods are to be derived. Different mechanisms of electrical stimulation (anodal/cathodal, make/break) will be modeled.

OBJECTIVES: To increase our understanding of the electrical properties of cardiac tissue, and to provide a mathematical model that can be used to study cardiac arrhythmias.

SIGNIFICANCE: Cardiac arrhythmias and fibrillation are two of the most common causes of death among Americans. In order to understand these pathologies, a basic knowledge of the heart and the electrical properties of cardiac tissue is required. In this study, we investigate idealized yet important models that reproduce many of the phenomena observed in cardiac tissue.

MAJOR FINDINGS: The intracellular and extracellular anisotropies influence the electrical properties of cardiac tissue. Tissues with "unequal anisotropy ratios" can lead to anodal stimulation and to arrhythmias generated by two pulses passing through a single electrode. Cardiac tissue is depolarized at the heart surface and deep within the tissue bulk by a uniform electric field.



The wave front evoked by successive stimulation from a point source (represented by the dot). Frames a) through d) show the wave front at sequential instants. The thicker curve corresponds to the depolarization phase of the wave front, the thinner curve to the repolarization phase.

PROPOSED COURSE: Analytical solutions to the bidomain equations using perturbation methods will be derived. Models of cathode-make, cathode-break, anode-make, and anode-break stimulation will be studied. The response of cardiac tissue to anodal stimulation will be examined, with an emphasis on explaining the "dip" in the anodal strength-interval curve.

PUBLICATIONS: Roth BJ. A mathematical model of make and break electrical stimulation of cardiac tissue by a unipolar anode or cathode. IEEE Trans Biomed Eng (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10387-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Three-Dimensional Motion Measurement System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Horace E. Cascio, BSEE Electronics Engineer EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
BL, RM, CC (S. Stanhope); Adtech (A. Dainis)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.50	0.40	0.10

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Biomechanics Laboratory makes quantitative information concerning human motion available to clinicians and researchers at the NIH. The BEIP has upgraded Dr. Stanhope's Vicon Motion and Image Analysis System by designing and fabricating six video-camera units, each with a variable intensity dual-wavelength infrared strobe. The upgrade solved many of the original Vicon system's problems, but fast motion analysis is still limited by the system's slow frame rate of 25 frames per second. The Biomechanics Laboratory requires a system to take data at 2000 frames per second. To satisfy this requirement, Dr. Stanhope requested that the BEIP develop a three-dimensional motion measurement system, using a concept patented by Dr. Andrew Dainis. The funds for this project are from a CRADA and have been placed with the BEIP. In collaboration with the Biomechanics Laboratory and Dr. Dainis, the BEIP will develop, fabricate, and test the system electronics. Dr. Dainis will develop the optics for the CCD linear arrays and the system software. The idea of Dr. Dainis's patent is that each camera has four linear CCD arrays, with each array having a specially designed lens. The arrays are placed on the face of the camera in a special pattern to translate a light source from a target into its spatial coordinates.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To design and fabricate a three-dimensional motion and image analysis system under a CRADA. The system will consist of several freestanding cameras, data acquisition and interfacing circuitry, and a controlling computer. A major goal of this project is to obtain a system with frame rates to 2000 Hz.

METHODS EMPLOYED: The system consists of one to sixteen cameras and a host system controller (personal computer). Each camera will contain an embedded computer that will control signal-processing capability, on-board memory, and interfacing circuitry to the host computer. All circuit-board designs will incorporate the new technology of Programmable Logic Devices (PLDs), recently acquired by the EEES. A single PLD, measuring only one inch square, contains hundreds of gates that may be configured to replace a logic circuit of many individual wired logic chips. The PLD circuit development is done on a PC and can be fully evaluated by the PLD programs. By using the PLD, circuit boards can be greatly reduced in size. Another advantage is that the PLD can be erased and reprogrammed when a system change is desired. The sensors are four 2048-element CCD linear arrays with a maximum sweep speed of 10 MHz. Frame rates near 5 kHz can be obtained, but a frame rate of 2 kHz would be acceptable. The cameras view targets located on a patient. The illumination of these targets can be active (LEDs or incandescent lamps) or passive (reflective spheres). The host computer controls the data acquisition session between all cameras. Once the session begins, the cameras will operate independently, releasing the host computer to perform other real-time functions, such as reading analog data from transducers attached to the patient's body. At the completion of the testing session, the PC reads, analyzes, and displays the data from each camera in an appropriate form. For a multicamera system with requirements to collect position coordinates of many targets at a fast frame rate and to collect real-time analog information, the use of parallel CPUs adds to system efficiency and reliability. The 486 processor in the host PC and a 386 processor on the embedded CPU card in each camera give this system dual processing capability.

SIGNIFICANCE: A three-dimensional motion analysis system that can perform studies at high frame rates would be a welcome addition to the Biomechanics Laboratory, especially in studies of lip and tongue motions.

PROPOSED COURSE: To design and fabricate one camera unit and the system controller. To test and evaluate the concept of the patent and the operation of the hardware that has been developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10388-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
3-D NMR Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ching-Nien Chen, Ph.D.	Physical Scientist	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews

_(b) Human tissues

X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new stereoscopic imaging technique was proposed and has been demonstrated. An RF probe and static gradients for a small test sample were constructed. Studies on phantoms and kiwi fruits have been completed.

PROGRESS: Images of phantoms and kiwi fruits have been obtained using this new stereoscopic method. A manuscript describing the technique is in preparation. An invited mini-symposium, entitled "Stereoscopic NMR Imaging," was given July 12, 1994. The presentation was sponsored by the Institute of Biomedical Sciences, Academia Sinica, Taiwan, and by the Veterans' General Hospital, Taipei.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10394-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Pulsed Electronic Spin Resonance System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas J. Pohida, MSEE	Electronic Engineer	EEES, BEIP, NCRR
Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
RBB, NCI (J. Mitchell, A. Russo, R. Murugesan, M. Krishna, S. Subramaniam); LCP, NIDDK (R. Tschudin)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
For several years, nitroxides have been a major focus of research in RBB, NCI, because of their importance to radiation biology in general and their potential utility for new photodynamic therapy techniques. Electron spin resonance (ESR) is a powerful tool for free radical studies, but in the past it has been of limited value for biological work (especially *in vivo*) because of the excessive attenuation by water of the radio frequencies ordinarily used, about 9 GHz. The system now being developed will operate at about one-thirtieth of that frequency to alleviate the attenuation problem, and will incorporate pulse techniques developed in NMR, as well as other new techniques, to compensate for the inevitable thirtyfold loss of sensitivity.

OBJECTIVES: To develop a pulsed ESR apparatus optimized for the study of nitroxides and other compounds of interest under *in vivo* conditions. Initial measurements will be spectroscopic, but imaging will be subsequently undertaken.

METHODS EMPLOYED: The initial configuration will comprise a set of Helmholtz coils, a probe, a pulse programmer, a radio frequency system, data acquisition and processing circuitry, and a PC for overall system control and data handling. New excitation techniques are under study to provide the broad bandwidth needed for nitroxides (approximately 100 MHz) without requiring excessive peak power. EEES personnel have designed and constructed a custom high-speed sampler/averager. Commercially available averaging units are not fast enough for these measurements--a limit imposed by the very short relaxation time of electrons compared to nuclei. This unit will sample and average 1000 free-induction decays, and will transfer the data to the computer in about 5 ms. The computer can further average 1000 such transfers, resulting in the averaging of 1,000,000 decays in about five seconds, with a thousandfold (or 60-decibel) improvement in signal-to-noise ratio, which will help compensate for the low sensitivity resulting from operation at a low frequency and other factors. In addition, EEES personnel have designed and constructed various radio-frequency components for the spectrometer. To enable averaging, for example, a multiple-frequency generator box was developed to provide a fixed-phase relationship (at each excitation) between all relevant frequencies. A narrowband transmit/receive (T/R) switch has also been delivered, although a broadband T/R switch will eventually be required. EEES has devised a method of constructing a broadband switch by introducing nontraditional transmission line impedance mismatches.

SIGNIFICANCE: Achievement of the proposed specifications will greatly facilitate research on nitroxides and other compounds of interest in biological systems.

PROPOSED COURSE: To implement the new broadband T/R switch. To design additional radio-frequency components as required by the spectrometer system. To provide complex signal processing algorithms for averaged data processing.

PUBLICATION: Pohida T, Fredrickson H, Tschudin R, Fessler J, Krishna M, Bourg J, Harrington F, Subramaniam S. High-speed digitizer/averager data acquisition system for Fourier transform electron paramagnetic resonance spectroscopy. Rev Sci Instrum 1994;65(8):2500-04.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10395-04 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Digital Differential Thermistor Thermometer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LBC, NHLBI (R. Berger)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
_(a1) Minors
(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Full exploitation of the potential of differential calorimetry requires small sensors with rapid response and high sensitivity, virtually limiting the choice to thermistors. The small size is necessary so that the sensors can be very close together to avoid masking a minute difference in heat of reaction by extraneous temperature gradients. For convenient use by laboratory and health-care personnel, the apparatus must be fully automated.

OBJECTIVES: To develop a fully automated differential thermistor bridge with high speed, sensitivity, and common mode rejection.

METHODS EMPLOYED: A personal computer (PC) with a standard plug-in interface board was used to provide the automation features, as well as data storage and display. A custom bridge configuration was designed to allow the computer to control balancing without the usual adjustment of the resistance of one arm, which is impossible with any standard interface board. The computer also controls bridge excitation to set the power level and allow sensing and correction of all thermoelectric potentials and amplifier offsets, as well as linear time variance of both.

SIGNIFICANCE: Achievement of the desired objectives will allow the construction and use of small probes with a suitable coating sensitive to the concentration of a specific compound on one thermistor. The instrument is also useful for general differential calorimetry, particularly when very fast response is needed.

PROPOSED COURSE: The instrument has been designed and built, and performs very close to the theoretical limits of speed and sensitivity imposed by the Johnson noise of the thermistors. It is now undergoing evaluation in several experimental applications. The development of specific compound probes will be undertaken soon. Circuit analysis has disclosed that departure from perfect balance affects thermal common mode rejection, so that a slight imbalance can be used to compensate for imperfect matching of the temperature coefficients of the two thermistors. If necessary, the software needed to effect this imbalance will be developed. A second unit with improved mechanical layout has been constructed. Software for more frequent measurement of common mode temperature was developed and will be used for several new applications. During the past year, the unit has been integrated into a stopped-flow system. All of the new software that was needed was developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10396-04 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Frozen-Hydrated Specimens by EELS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR
Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

LN, NINDS (S. B. Andrews, T. S. Reese)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
    (a1) Minors
    (a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The water-mapping technique that we developed, based on electron energy-loss spectroscopy (EELS) in the scanning transmission electron microscope (STEM), has been developed further to map subcellular distribution of water in frozen-hydrated biological cryosections. Previously, methods for water determination were indirect in that they required the cryosections to be dehydrated first. The new approach makes use of spectrum-imaging, whereby EELS data are collected in parallel at each pixel. Several operations are required to process the spectra, including subtraction of the detector dark current; deconvolution by the detector point-spread function; removal of plural inelastic scattering; and correction for the support film. The resulting single scattering distributions are fitted to standard reference spectra at each pixel, and water content can be determined from the fitting coefficients. Although the dark-field or bright-field image from a hydrated cryosection shows minimal structure, the processed EELS image reveals strong contrast due to variations in water content.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To develop a direct method, based on scanning transmission electron microscopy and electron energy-loss spectroscopy, for mapping subcellular water distributions in frozen-hydrated tissue quantitatively.

METHODS EMPLOYED: Frozen cyrosections of rapidly frozen liver were transferred into a VG Microscopes HB501 scanning transmission electron microscope (STEM) operating at a beam energy of 100 keV and equipped with a parallel-detection EELS. By operating with a reduced current into a defocused probe approximately 50 nm in diameter, EELS digital spectrum-images were acquired at low electron dose, with total acquisition times of around 1000 s. Processing techniques were developed for: (i) subtracting the detector dark current, (ii) deconvoluting the detector point-spread function, (iii) deriving the single scattering intensity, (iv) correcting for the support film, and (v) fitting reference spectra from ice and organic components, using a multiple least squares algorithm.

MAJOR FINDINGS: Water distributions can be obtained by analyzing electron energy-loss spectrum-images recorded at low electron dose from frozen hydrated cryosections. Doses below about 2000 electrons per square nanometer do not appear to produce significant alterations in the energy-loss spectra from cryosections of thickness around 200 nanometers. Under our experimental conditions, the spatial resolution of the technique at this dose is limited to 80 nanometers. This scale of measurement is useful because it is commensurable with the cryosection thickness. It has been found that the valence electron spectrum from most subcellular compartments can be modeled within a reasonable approximation by spectra from water and protein. Greater accuracy should be achievable in some specimens by applying the fitting procedure iteratively and by modifying the reference spectrum for the organic components in specific identified organelles to provide a more realistic composition. A precision of 2% has been demonstrated for the measurement of mitochondrial water content; and because some of this scatter may be due to biological variability, the actual precision in the measurement may be nearer to 1%. The absolute accuracy of the method for most subcellular compartments is estimated to be 3%, as limited by uncertainty in choice of reference spectra and the MLS fitting parameters.

SIGNIFICANCE: The water content of compartments in the cell is an important physiological quantity that reflects how fluid and electrolyte concentrations are regulated, both indirectly (by osmotic effects following ion movements) and directly (through water-specific channels). Changes in water content may accompany important cellular events, e.g., secretion. In addition, changes in water distribution can indicate pathological states of the tissue, such as the swelling of neuroglia after brain injury. The new EELS water-mapping technique provides a means of obtaining direct water measurements in cells at below 100 nm spatial resolution for the first time.

PROPOSED COURSE: We plan to improve the sensitivity of the water measurement by utilizing a slow-scan charge-coupled device (CCD) with a higher detective quantum efficiency at low counting rates. We expect that the water-imaging approach will be useful in a wide range of biological applications aimed at determining physiological or pathophysiological water distributions on the subcellular scale.

PUBLICATIONS: Sun SQ, Shi SL, Andrews SB, Leapman RD. Imaging water distributions in frozen-hydrated hepatocytes by EELS. In: Friel JJ, ed. Proceedings of the 28th annual MAS meeting. New York: VCH Publishers, 1994;117-18.

Sun S, Shi S, Hunt JA, Leapman RD. Quantitative water mapping of cryosectioned cells by electron energy-loss spectroscopy. J Microsc 1995;177:18-30.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10398-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diffusion Tensor Spectroscopy and Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Peter J. Basser, Ph.D. Biomedical Engineer MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LCE, NHLBI (P. Jezzard); NB, NINDS (G. DiChiro, C. Pierpaoli, A. Barnett)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892-5766

TOTAL STAFF YEARS: 0.85	PROFESSIONAL: 0.85	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been developing a new MRI modality that we call Diffusion Tensor MRI. It consists of a) estimating an effective diffusion tensor, $\underline{D}$, in a voxel from a series of diffusion-weighted images, and b) using $\underline{D}$ to derive useful information about tissue structure and function. We produce images of diffusion ellipsoids that depict local fiber orientation and mean diffusion distances, as well as images of scalar invariants of $\underline{D}$ that are independent of the reference frame in which magnetic field gradients and $\underline{D}$ are measured. *Quantitative* Diffusion Tensor MRI is what we call the calculation and display of MR imaging parameters that behave like histological or physiological stains. These include "stains" for diffusion isotropy, diffusion anisotropy, structural similarity, and fiber-tract organization.

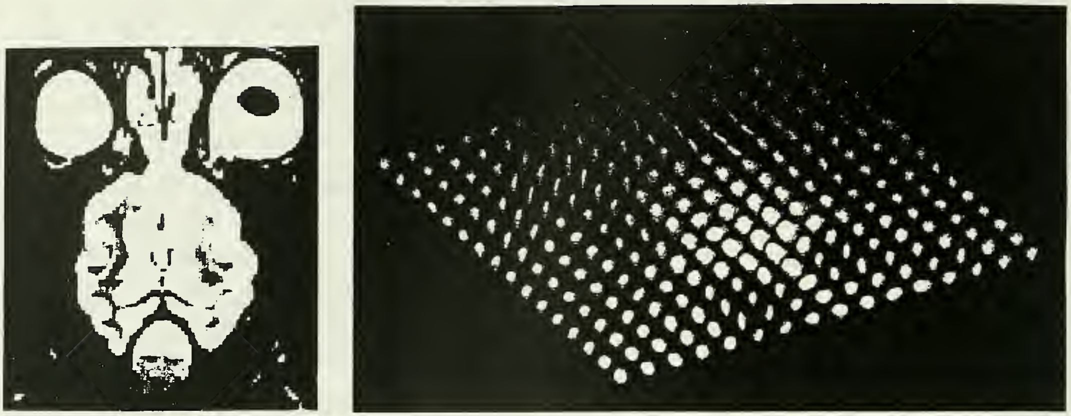
To account for the complex interactions between imaging and diffusion gradient pulses applied during the acquisition of diffusion-weighted images, we have derived analytic expressions of the "b-matrix" for general 2-D FT and EPI diffusion-weighted sequences. These b-matrices are calculated for each diffusion-weighted image in order to estimate $\underline{D}$ off-line. More recently, we have developed b-matrices for ultra-fast sequences (e.g., interleaved EPI) that allow Diffusion Tensor Imaging to be performed at a sufficiently high resolution and speed for radiological assessment in a clinical setting.

OBJECTIVES: To increase our understanding of the microscopic environment in tissues by measuring the displacement distribution of water or metabolite molecules. To develop clinically viable methods to measure displacement distributions of protons or metabolites. To use these methods in studies of normal physiological processes, disease, development, and aging.

SIGNIFICANCE: Other than MR, no noninvasive method exists to measure water or metabolite mobility *in vivo*. Using the effective diffusion tensor, $\underline{D}$, we have the capability to infer changes in local microstructure (e.g., fiber orientation, membrane permeability, microanatomy, etc.), as well as to monitor microdynamic changes (e.g., in viscosity). Moreover, in anisotropic tissue (e.g., in brain white matter and skeletal muscle), we have pioneered the use of invariant quantities (e.g., the Trace of $\underline{D}$) to provide structural and functional information independent of tissue orientation. These scalar invariants are intrinsic to the tissue and independent of its orientation within the MR magnet. They are readily measured and monitored in normal physiological processes, disease, development, and aging.

MAJOR FINDINGS: The effective diffusion tensor, $\underline{D}$, has been estimated for isotropic and anisotropic media *in vitro* and *in vivo*. We have successfully inferred fiber direction in skeletal muscle and white matter noninvasively to within less than one percent, and we have proposed and demonstrated the potential utility of invariant quantities, such as the Trace of $\underline{D}$, as MR imaging parameters. We showed that significant errors are made in diffusion MR spectroscopy and imaging of anisotropic media when off-diagonal elements of $\underline{D}$ are not considered. More recently, Dr. James Mattiello (formerly a Staff Fellow in BEIP) calculated b-matrices for generalized 2-D FT and EPI diffusion sequences, thus permitting the rapid estimation of the diffusion tensor from diffusion-weighted images. This activity has been continued by Dr. Carlo Pierpaoli, who has developed b-matrices for generalized diffusion-weighted, interleaved EPI sequences previously developed by Dr. Peter Jezzard. These expressions give us the ability to estimate $\underline{D}$ from series of diffusion-weighted images by accounting for the complex and multiple interactions between imaging and diffusion gradients in both the parallel and the perpendicular directions.

Our group was the first to demonstrate Diffusion Tensor Echo-Planar Imaging (DTEPI) of live human brain and to establish its clinical feasibility. This required us to obtain a large number of diffusion-weighted images, free of motion artifacts, in a few minutes' time. Recently, we combined the estimation of $\underline{D}$ with a rapid imaging technique, Interleaved Echo-Planar Imaging (EPI), which allowed us to monitor certain processes (e.g., thermal conduction and molecular diffusion) *in vivo* without observable motion artifacts.



a) b)

a) T2-weighted image of living monkey brain. b) The corresponding effective diffusion ellipsoid image of a region of interest containing the 3rd ventricle.

PROPOSED COURSE: To continue to explore the benefits and potential uses of Diffusion Tensor MRI and MR spectroscopy in both biomedical and clinical research.

PUBLICATIONS: LeBihan D, Basser PJ. Molecular diffusion and nuclear magnetic resonance. In: LeBihan D, ed. Diffusion and perfusion magnetic resonance imaging. New York: Raven Press, 1995;5-17.

Basser PJ, LeBihan D, Mattiello J. Anisotropic diffusion: MR diffusion tensor imaging. In: LeBihan D, ed. Diffusion and perfusion magnetic resonance imaging. New York: Raven Press, 1995;140-9.

Basser PJ, Pierpaoli C. Diffusion Tensor MRI: a new method to elucidate tissue structure and organization. In: Proceedings of the 1995 Bioengineering Conference. Beaver Creek, CO: 1995;29:367-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10399-03 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of a Cell Sorter

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John I. Peterson, Ph.D.	Chemist	BEIP, NCRR
Stephen B. Leighton, Ph.D.	Mechanical Engineer	BEIP, NCRR

COOPERATING UNITS *(if any)*

MET, DCBDC, NCI (J. Berzofsky)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Chemical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An instrument is being developed that will be able to select individual cells from growth medium and place them in wells in a tray (or some other container) for further testing or monoclonal growth. Traditional pipetting of a diluted cell suspension is slow and labor-intensive, and requires further processing to select samples containing a single cell. The instrument under development would combine automatic cell selection with computer-controlled cell placement.

PHS 6040 (Rev. 5/92)

OBJECTIVES: The objective of this project is to develop an instrument that can select single cells from a growth medium and place them into wells on a plate. The instrument would be based on a flow system in which the cells pass through a detector that would trigger their selection and placement.

METHODS EMPLOYED: To determine the feasibility of optical detection of cells.

SIGNIFICANCE: The selection of single cells by pipetting them into plate wells from a very dilute medium is slow and tedious, and is based on a statistical expectation of getting mostly single cells in a selection. Further testing and evaluation must be done to discard wells with either no cells or more than one cell. The instrument planned would be completely automatic in cell selection and placement, and would be of considerably greater utility than a commercially available instrument that places cells robotically but requires visual-manual selection.

MAJOR FINDINGS: A system had been developed previously that was based on electrochemical detection of cells, using the "Coulter counter" principle of measuring the increase in electrical resistance through an orifice as a cell passes through it. This approach to detection could not be made to work satisfactorily.

An alternate approach, that of using optical detection of the cells, is now being tested. One of the requirements is that the cells cannot be altered in any way, such as by tagging with dyes, so the feasibility of detection of the cells by their normal optical properties is being explored. The system must be able to distinguish single cells from groups of cells and from non-cell material.

The plan was to design a detector with a small laser source, a lens arrangement, a flow capillary, and an array detector with computer discrimination. It was found that optical cell detection is entirely practical, with good signal-to-noise characteristics. It is not yet clear whether the optical arrangement is optimal, although its simplicity is attractive.

PROPOSED COURSE: Work will continue to determine the optimum optical arrangement for cell detection, and to test and develop an associated flow system for cell selection. It will then be necessary to combine the optical detection method with a computer-based discrimination system for recognizing single cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10403-04 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional Analysis and Applications to Biomedical Image Processing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR
Michael Unser, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
[✓ (c) Neither]

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An important aspect of biomedical data and image processing is to find signal representations that are adapted to the application. The Fourier transform, for example, is well adapted to analyzing the frequency components of signals. Shannon's sampling theory is well adapted to analog/digital conversions, and is therefore well suited to the processing and storage of signals and images by digital computers. However, Shannon's sampling theory is restricted to bandlimited functions. We have developed a general sampling procedure for non-bandlimited signals. In particular, by considering sampling as a problem of approximation in translation-invariant function spaces, we have shown that the least squares approximation of a signal consists of a sampling procedure generalizing the classic one. It consists of an optimal prefiltering, a pure jitter-stable sampling, and a postfiltering for the reconstruction. We have also developed a general theory of representation that uses the new concepts of multiresolutions and wavelets, which are well adapted to multiscale signal processing, edge-detection tasks, signal analyses, coding, and compression.

OBJECTIVES: Our goal is to develop transforms that are well suited to different tasks of data processing and biomedical image processing. These transforms should be adapted to problems in signal analysis, such as EEG or ECG signals. They should also be useful for detection tasks and for storage and retrieval of large volumes of data, such as MRI images, image series, and 3-D images.

METHODS EMPLOYED: The sampling theory is based on the study of function spaces obtained by weighted sums of a generating function and its translations on the integers. The orthogonal projection of a signal on these spaces is equivalent to a sampling procedure, which consists of a prefiltering followed by a sampling and a postfiltering for the reconstruction. The multiresolution and wavelet theories of representation are similar to the sampling theory in that they are defined using a single function. The linear combination of the translations and dilations of the scaling function generates the multiresolution signal spaces. A similar construction with a wavelet function generates the wavelet spaces. By using convolution of scaling functions, we obtain other scaling functions with more smoothness. Using linear combination of the translations of a scaling function (or wavelet), we construct other scaling functions (or wavelets) with certain desired properties.

MAJOR FINDINGS: The sampling theory we have derived generalizes Shannon's sampling procedure to non-bandlimited signal spaces. Moreover, we have established the link with Shannon's sampling theory. The convolution sequences of generating functions indexed with n allow us to construct sequences of increasingly smooth signal spaces. We have proven that the corresponding sampling schemes converge to the classic sampling procedure of Shannon as the index n tends to infinity. We have found a simple method for constructing scaling and wavelet functions with some desired properties. For example, we can construct symmetrical interpolating wavelets that are as smooth as we want, and that have as good bandpass characteristics as we choose. We can also construct wavelets with optimal time-frequency localization, starting from any given wavelet function.

SIGNIFICANCE: This work will provide the scientific community with new tools for the analysis and processing of data and images. The sampling theory has already been applied to the design of new tomographic reconstruction algorithms. It has also been applied to the enlargement and reduction of images. The multiresolution and wavelet representations are currently being applied at the NIH to image coding, image transmission, and the rotational and translational alignment of autoradiograms.

PUBLICATIONS: Aldroubi A. Portrait of frames. Proc Amer Math Soc (in press).

Aldroubi A. Oblique projections in atomic spaces. Proc Amer Math Soc (in press).

Unser M, Aldroubi A. A general sampling theory for nonideal acquisition devices. IEEE Trans Signal Process 1995;42:292-5.

Woderman H, Aldroubi A. Extrapolation in multiresolutions. In: Laine A, Unser M, eds. Mathematical imaging: wavelet applications in signal and image processing. 1994;2303:120-8.

Aldroubi A, Unser M. Oblique projections in discrete signal subspaces of l_2 and the wavelet transform. In: Laine A, Unser M, eds. Mathematical imaging: wavelet applications in signal and image processing. 1994;2303:36-46.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10404-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical Methods in Gel Electrophoresis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR
Michael Unser, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
LTPB, NICHD (M. Garner, A. Chrambach); DCRT

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects (b) Human tissues X (c) Neither
_(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Electrophoresis is a technique widely used in biology to separate, classify, and study complex mixtures of macromolecules and particles (such as DNA). We have applied mathematical methods and techniques to problems in electrophoresis. In particular, we have developed models describing the motion of macromolecules and virus-sized particles in gels. We have defined a resolution function that is well adapted to electrophoretic separation of particles such as DNA. The models of motion, together with the resolution function, allow us to predict the minimal time and optimal migration path for the resolution of complex mixtures, such as those encountered in DNA sequencing. We have also developed methods for evaluating the efficiency of various polymers in the separation of macromolecules of various sizes. For this purpose, we have defined the separation efficiency function, as well as the resolution efficiency function. These functions are used to evaluate different polymers relative to one another. These techniques give us a practical means by which to select the most efficient sieving polymer solution for a particular molecular size. Finally, we have implemented the mathematical algorithms used in these analyses in a user-friendly software package for use on a personal computer.

OBJECTIVES: Our goal is to develop models, techniques, and methods for the quantitative analysis of gel electrophoresis. More specifically, we want to develop means by which to predict how different particles separate in various media.

METHODS EMPLOYED: The motion of the particles is described by combining Ogston's model of gel electrophoresis with the diffusion equation in partial differential equations. The resolution function is derived from a property of Gaussian functions. The time and migration path of particles in gels are deduced by combining the equations of motion with the resolution function. The efficiency function is deduced by using Ogston's model of particle mobility in gels for two particles with different radii, and letting the difference tend to zero.

MAJOR FINDINGS: Our models describe the motion of macromolecules and virus-sized particles in gels. The resolution function we have defined describes the degree of separation of a mixture of particle populations. The equations we derived predict the time length needed for separating a mixture under experimental conditions. The resolution efficiency function we have found allows us to compare the effectiveness of different media in sieving both large and small particles. Our theory has been applied to calculate the capillary electrophoresis of DNA sequencing separations. It showed that acceptable resolution could be obtained using shorter times than those commonly used.

SIGNIFICANCE: A savings of time (by a factor of about 2.5) can be achieved in the separation of DNA mixtures. Thus, with our method, we can minimize the electrophoresis time in DNA sequencing, circumventing one of the limiting steps in electrophoresis. We have also applied our theory to evaluate four polymers relative to one another in terms of their efficiency in the separation of macromolecules of various sizes, and derived a nomogram that allows the practical selection of the sieving medium and the conditions for the separation of molecules of a desired size range.

PUBLICATIONS: Aldroubi A, Zkharov SF, and Chrambach A. A computer program for predicting recovery of SDS-protein in the automated HPGE-1000 apparatus. Appl Theor Electrophor (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10405-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A System to Measure Head Motion Inside a PET Scanner

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Seth Goldstein, Sc.D. Chief MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
NM, CC (M. D. Witherspoon, M. Green)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.15	PROFESSIONAL: 0.15	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An optical system has been developed to measure the small motions of a person's head during a positron emission tomography (PET) scan in order to correct the scan and thereby improve the resolution of the image. The system uses two position-sensitive detectors to sense changes sequentially in the positions of three blinking miniature lamps that are fixed to the patient's head. The computer-controlled instrument computes the six degrees of freedom (three angles and three positions) that are necessary to characterize head movement.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To improve the resolution of PET scans, it is necessary either to eliminate head motion or to correct for it. Since methods to eliminate head motion have been inadequate to date, the present system has been designed to measure head motion so that corrections can be made to the scan.

SIGNIFICANCE: The effective resolution of PET scans (~5 mm) is degraded by approximately a factor of 2, due to the effects of head motion during a scan and between two scans taken an hour apart. Elimination of this additional uncertainty in measurements would represent a significant advance. The cost of the system under development is only a few percent of the scanner's cost. Its use should put a minimal additional burden on the system operator, since it is under the control of user-friendly software.

METHODS EMPLOYED: Patient head motion is determined by optically measuring the positions of three blinking miniature lamps fixed to the head, using two position-sensitive detectors (PSDs). These devices sense the x- and y-coordinates of the centroid of the light falling on their photosensitive surface. By using sequentially blinking, extremely bright lights under stable, relatively dark ambient conditions, the PSD output can be related unambiguously to the lamp position. The sensor data acquisition, is controlled by a Macintosh computer running the program Labview. This program does the required computations to generate the three angles and three positions required to specify head motion. The computer also controls the instrument setup, thus relieving the technician of this burden.

MAJOR FINDINGS: The previously developed system utilizing GPIB-controlled electronics has been replaced with software and electronics developed for a related project to measure motion inside an MRI scanner. The new system provides a position update every ~0.7 second. A variety of measurement artifacts, caused by small amounts of light from the blinking lamp that reflected off of adjacent surfaces, were eliminated by masking the illumination at the bulb and placing a light shield around the entire lamp assembly. Final checkout, which involved comparing the sensor readings with a commercially available electromagnetic system (which does not function in the PET environment), indicates system agreement when using a mechanical fixture and when using patients.

PROPOSED COURSE: The system will be installed in a PET scanner for trials with phantoms in order to test the software that will be used to correct images taken in the presence of motion. It will then be used with patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10406-04 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of a Fiber-Optic Gastric pH Sensor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John I. Peterson, Ph.D.	Chemist	CHES, BEIP, NCRR
Eden J. Netto, M.Sc.	Guest Researcher	CHES, BEIP, NCRR
Victoria A. Hampshire, D.V.M.	Chief	CUUB, VRP, NCRR

COOPERATING UNITS *(if any)*
DD, NIDDK (R. T. Jensen)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A fiber-optic pH sensor and associated instrumentation were developed for measurement and monitoring of intraesophageal and intragastric pH. Composed of replaceable sensors, an optical system, and electronic circuitry, the instrument measures pH in the range of 0-7, with an accuracy of 0.1 pH units and a response time in the range of 30 sec, for up to 24 hours. This instrument should be useful for the research, diagnosis, and evaluation of acid-related disorders of the upper gastrointestinal tract, and for studies on the influence of diet and antisecretory drugs.

OBJECTIVES: The objective of this work is to develop a dye-based sensor that covers a broad pH range from neutral to high gastric acidities. Several fiber-optic pH sensor systems have been developed, based on extensions of the approach originated here by Drs. Peterson and Goldstein; but with only one exception, they have not been for the low pH range. (The exception is a system intended for very high acidity measurements in nuclear fuel technology.) In addition, the few published methods for extending the pH range have not been suitable for this application.

METHODS EMPLOYED: The project consisted of several areas of development:

1. A system of dyes was developed and optimized for the correct optical and chemical characteristics (acidity constants) to cover the pH range of interest and to provide suitable wavelength and absorbance properties.

2. This system was combined with a dye-support system having several goals: (a) to fix the dyes, so they will be stable; (b) to provide a diffusion medium, so they will equilibrate with the surrounding pH; and (c) to be formed into a probe system with a short response time.

3. A probe arrangement was devised to be physically strong and to provide a container for the dye system that allows diffusion of hydrogen ions while limiting the flow of higher-molecular-weight substances.

4. Instrumentation was developed that combined an optical system with the probe and dye system. An instrument was constructed for testing the performance of the pH probes. The instrumentation included a CCD/grating spectrometer and a computer program written for data processing and control.

5. The system was tested and its performance was evaluated *in vitro* in gastric fluid. No interference from colored substances, possible destructive action on the probe, or ionic strength error was observed.

6. A test was done, using a dog as the subject, to evaluate the *in vivo* performance of the system in comparison with that of a gastric pH electrode.

SIGNIFICANCE: This project represents a significant contribution to biomedical engineering and clinical research, since current techniques involving electrodes are uncomfortable, cumbersome, and subject to difficulties due to electrode coatings and electrical safety hazards. A suitably designed fiber-optic probe would be smaller, safer, and more reliable, and would facilitate diagnostic and research measurements with a patient-wearable system. The small size expands the possible clinical and veterinary applications.

MAJOR FINDINGS: The system has been developed and validated. The original design goals were achieved.

PROPOSED COURSE: It would be desirable to do further testing of the system's performance *in vivo,* and to develop the instrumentation further in the form of a portable or wearable unit for use in clinical evaluation of people or animals. There is also some interest in a distributed sensor system that would measure pH at several locations along the fiber-optic lead.

PUBLICATIONS: Netto EJ, Peterson JI, McShane M, Hampshire VA. A fiber-optic broad-range pH sensor system for gastric measurements. Sensors & Actuators B (Chemical) 1994;29(1-3):157-63.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10407-04 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Elasticity and Active Force Generation in Cochlear Outer Hair Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Chadwick, Ph.D.	Head, Biomech. Group	OD, BEIP, NCRR
Emilios Dimitriadis, Ph.D.	Biomech. Group	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

LCB, NIDCD (K. Iwasa)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.6	PROFESSIONAL: 1.6	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the epsce provided.)*

To establish the magnitude of the electromechanical force generated by the outer hair cell from the mammalian cochlea, we used a cylindrical membrane model characterized by area and shear moduli for a passive elastic element, and a membrane potential-dependent, active tension-generating element. We measured pressure-strain relations to determine the elastic moduli, and found the area modulus was both close to a lipid bilayer and an order of magnitude larger than the shear modulus. We also determined that the active tension element is nearly isotropic, with an amplitude sensitivity of about $2x10^{-2}$ $Nm^{-1}V^{-1}$. At 73 dB of acoustical stimulation, the active force generated per outer hair cell is about 0.6 nN--close to the force applied to a corresponding area of the basilar membrane by acoustic pressure. This finding supports the hypothesis that the outer hair cell acts as a feedback motor in the fine-tuning mechanism of the mammalian ear. A model has been developed to study outer hair-cell feedback in waves on the cochlear partitions and the resulting sharpening mechanism.

PHS 6040 (Rev. 5/92)

OBJECTIVES: The motility of the outer hair cell (OHC) from the mammalian cochlea is considered essential for fine tuning. Extensive efforts have been directed toward characterizing the motility mechanism. It has been established that it is membrane potential-dependent. A hyperpolarizing pulse elongates the cell, and a depolarizing pulse shortens it. Tight-sealed membrane patches, formed on the lateral wall, undergo area changes when the membrane potential is changed. A hyperpolarizing pulse increases membrane area, and a depolarizing pulse reduces it. Although these electrokinetic shape changes are well characterized, it has not been previously demonstrated that an OHC can generate sufficient force to influence the basilar membrane. We use a biomechanical membrane model of the OHC to investigate this issue. The influence of OHC force and elasticity on sharpening the waves on the cochlear partition is being investigated.

METHODS EMPLOYED: Isolated outer hair cells from guinea-pig cochlea, light microscopy, inflation by micropipette, osmotic swelling, mathematical modeling, and image analysis are used in this project.

SIGNIFICANCE: The magnitude of the active force is comparable to that due to acoustic loading. Hence, the OHC has sufficient strength to act as a feedback motor for fine tuning of the mammalian cochlea.

PROPOSED COURSE: Capacitance measurements are being carried out to further assess the magnitude of OHC surface area changes. Experiments are planned to characterize the dynamic mechanical response of the OHC.

PUBLICATIONS: Iwasa KH, Chadwick RS, Dimitriadis EK. Membrane-based motor motility of the outer hair cell and its function. In: Frontiers of biological physics. Am Inst Physics, 1994 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10410-04 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

MRI Muscle Dynamometer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen B. Leighton, Sc.D.	Mechanical Engineer	MES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electrical Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

IR CE, NHLBI (T. Ryschon)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Mechanical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects
  (a1) Minors
  (a2) Interviews

(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is useful to be able to make *in vivo* metabolic measurements (e.g., of muscle phosphocreatinine) with magnetic resonance spectroscopy while subjects are exercising specific muscle groups on a dynamometer. It is the purpose of the present work to design and construct a programmable wrist- and ankle-joint dynamometer for use in the magnetic environment of the NIH 4-Tesla MRI instrument.

PHS 6040 (Rev. 5/92)

OBJECTIVES: Human studies of muscle energy metabolism during exercise have traditionally relied on chemical analysis of muscle samples obtained immediately following a trial of muscular work. Such tissue samples are acquired through a percutaneous approach to an easily accessible muscle mass, and must be limited in number to avoid functional disruption of the muscle. In addition, ethical considerations have precluded using this technique to study children's muscles. ^{31}P-NMR spectroscopy offers a noninvasive alternative to muscle biopsy for the measurement of high-energy phosphate concentration (ATP, Pi, ADP, PCr). In addition to being noninvasive, measurements can be acquired during exercise, and several parameters can be measured simultaneously, giving greater insight into the energetic processes involved in muscle contraction. Meaningful studies of muscular work require careful control and measurement of muscle tension. Bicycle ergometers, treadmills, and kinetic dynamometers are commonly used in the exercise physiology laboratory, but cannot be used in the NMR experiment for several reasons. The high-strength (4-Tesla) magnetic field required for NMR measurements precludes the use of any ferrometallic devices within 10 to 12 feet of the magnet. NMR measurements require the muscle to be placed in the center of the magnetic field, which is found midway inside the cylindrical bore of the magnet (5 meters in length and 70 cm in diameter). Thus, the dimensions and composition of any ergometer for use in NMR studies of humans must be unique, and cannot be satisfied by commercially available devices. An ergometer has been designed and constructed by the BEIP to meet these critical specifications. The ergometer will allow use with children--a group that has not been adequately studied. The first implementation of the ergometer will be to study the high-energy phosphate concentration of contracting calf muscle in children at different stages of maturity and at different levels of physical conditioning. For this study, a pedal will be driven by the main ergometer, allowing precise control of the resistance to ankle flexion and extension, and precise measurement of the force produced in opposing this resistance. In subsequent studies, an arm module will be implemented to study the energetics of flexion and extension of the wrist. The ergometer will provide state-of-the-art, unprecedented control of the exercise being investigated.

We intend to create a system that is able to subject the ankle or wrist joints to programmed torques, position functions, or velocities. Magnitudes of the parameters will be adjustable to either adult or pediatric subjects. Torques will either resist or abet muscle motion, allowing both eccentric and concentric work to be studied. All parts of the system within the MRI magnet will be nonmagnetic, in order to avoid any interference with the spectroscopic measurements. All system controls and all logging of data will be handled by a remote personal computer.

METHODS EMPLOYED: A DC servo motor system is mounted on a cart positioned on the axis of the MRI coil, about 3 meters from the end of the coil. At this location, the magnetic field is weak enough that the servo system is still functional. Likewise, the iron and working fields of the servo system do not affect the operation of the MRI instrument. The output of the motor is impedance-matched to the load inertia with a 16:1 gearhead, and converted to nearly linear motion with a simple crank. A 5-meter-long rigid thin-wall tube structure acts as a push-pull link to connect the motor crank to the patient pedal within the magnet. The tube structure is aluminum, up to the magnet and fiberglass-reinforced epoxy composite within the magnet coil. An identical but stationary tube connects the motor base (cart) to the patient pallet so that reaction forces do not disturb the magnet or move the motor cart. A "panic button" and quick-release system allow the subject to be removed from the magnet on demand at any time.

Ankle-joint torque is monitored by a unique fiber-optic torque sensor, since electronic signals would be contaminated by artifacts from the motion in the magnetic field. An improved version of this sensor has been incorporated that uses a multi-leaf beryllium copper spring as a linear strain element with less hysteresis than in the previous composite torque arm. This element is incorporated in series, with the push-pull link attached to the pedal crank. The multifiber optical bundle from the previous sensor faces a reflecting surface across the gap in the spring element. Increasing torque increases the gap and decreases the amount of light reflected from the light-delivery fibers in the bundle back to the light-receiving fibers in the same bundle. Electronic transduction of this signal is carried out well away from the MRI field.

The servo loop is closed by a commercial digital controller with a 1-KHz bandwidth, which accepts incremental encoder signals from the motor and sends commands to a 10-KHz-bandwidth switched-mode amplifier operating in a closed-velocity loop with a linear tachometer (also mounted on the motor). The controller accepts position commands downloaded from a personal computer (PC). The PC carries out data-logging tasks and overall system control. The controller may also be put in a torque mode, in which it compares the torque signal from the fiber-optic sensor to a desired torque waveform.

A second interface unit has been added that contains an embedded PC. This unit communicates with the main controlling PC via an RS232 link, and provides all of the analog and digital I/O for the system. With this embedded PC, the system is able to perform this function at a much faster rate than was previously attainable with the motorized controller. In addition, a second digital shaft encoder has been added that connects directly to the main controlling PC. These two additions allow the acquisition of shaft position, velocity, and torque at rates of up to 5 milliseconds per data point, so that finer structure of the test subjects' responses may be measured.

MAJOR FINDINGS: The apparatus continues to perform satisfactorily with human subjects, including pediatric patients. For work with the weakest subjects, some residual hysteresis (less than 0.5 ft-lbs) stills needs to be removed.

SIGNIFICANCE: This apparatus will permit new studies of *in vivo* human muscle metabolism during specific exercise regimens, for eliciting new information on muscle mechanics and biochemistry.

PROPOSED COURSE: Nonmagnetic roller bearings will be substituted for some existing bushings in order to reduce the remaining hysteresis. Additional paradigms, such as the torque and power modes, will be fully exploited in further experiments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10413-03 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Female Rhesus Monkey Reproductive Physiology and Behavior Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas J. Pohida, MSEE	Electronic Engineer	EEES, BEIP, NCRR
Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LCE, NICHD (E. Phoebus)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dr. Phoebus has a 7-channel commercial telemetry system, which he plans to implant in monkeys in order to monitor several physiological parameters. He wants to use a channel originally intended for EEG signals to monitor intrauterine pressure. An interface will be required between the pressure transducer and the FM telemetry system. Switchable battery power, also to be implanted, is necessary to provide an adequate system-life time after implantation. During system operation, general system support will be necessary and receiver data-processing hardware will need to be developed.

OBJECTIVES: To design and construct six complete systems. The system design must provide high sensitivity, consume a minimal amount of power, be of minimal physical size, and sustain long-term implantation.

METHODS EMPLOYED: The system will consist primarily of two packages. The first package will include the commercial FM transmitter, the pressure transducer, and custom interface circuitry. EEES personnel designed the interface circuitry, which FM-modulates the pressure signal prior to input into the EEG channel. The design consumes less than 1 mA and is constructed with surface-mount components to maintain minimal size. The second package includes batteries and an IR switch, which can be used to turn the system power off when data collection is not desired.

SIGNIFICANCE: The proposed telemetry system will acquire data that will be used to establish a biomedical animal model of female anorgasmia, which will enable Dr. Phoebus to study the effects on sperm migration, developmental factors in anorgasmia, and social organization.

PROPOSED COURSE: To complete construction of six systems, which includes the electrical assembly and the packaging of the electronics for implantation. (Testing will be performed prior to implantation.) To provide system support during data collection. To design an FM discriminator to extract pressure data from the 7-channel demodulator output.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10414-03 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
C-13 MRS Studies of Metabolic Paths and Conversion of Ibuprofen Enantiomers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ching-Nien Chen, Ph.D. Physical Scientist OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
Rhode Island University (C-S. Chen); Howard University (P. C. Wang)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ibuprofen is an anti-inflammatory drug. This activity is based on its ability to inhibit the function of cyclo-oxygenase. Ibuprofen exists in two enantiomeric forms because of an asymmetric center alpha to the carbonyl group. *In vitro* studies have shown that ibuprofen is only active in the S-form enantiomer; conversion of the inactive R- to the active S-form *in vivo* has been postulated and supported by *in vitro* evidence. Previously, the study of ibuprofen metabolism was done by invasive means. Our approach is noninvasive and *in vivo*, using live rats in an NMR imaging instrument. The ibuprofen enantiomers are labeled at the C2' position with 13-C and 2-H, and the drug's 13-C NMR spectrum variations were followed from the liver region of the rat.

METHODS EMPLOYED: We studied the uptake and conversion of ibuprofen in rat liver by following the NMR C-13 signals of the asymmetric carbon center, C2. The R- and S-ibuprofen enantiomers and the R-form of the ibuprofen-CoA thioester intermediate were synthesized and enzymatically separated. R- and S-[2-13C,2-2H]-ibuprofen were administered to the anesthetized animal, and the time course of the C-13 signals were recorded. A superconducting magnet operating at 4.7-T was used. A solenoidal coil around the rat was used as the RF transmitter/receiver.

PROGRESS: A poster presentation and several oral presentations describing this work have been given.

PUBLICATION: Chen C-N, Wang PC, Song HF, Liu YC, Chen CS. The role of NMR in the study of a drug structural inversion. In: Proceedings of the 9th Conference on Magnetism & Magnetic Technologies. 1994;347-52.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10416-03 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical Modeling of Calcium Waves in Astroglia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bradley J. Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LMGD, NICHD (J. Russell)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium-dependent fluorescent dyes have been used to observe calcium waves in astroglia. Mathematical models exist that describe the behavior of these calcium waves in time, but their combined temporal/spatial behavior is unexplored. The goal of this project is to develop a model, similar to reaction/diffusion models for other excitable media, that is consistent with the experimental data. This model will be examined using computer simulations and emphasizing qualitative predictions that can be tested experimentally.

OBJECTIVES: To increase our understanding of calcium waves in astroglia, and to provide a mathematical model that can be used to describe these waves.

SIGNIFICANCE: The function of glial cells in the nervous system is not known, but they may play a role in the slow responses of the nervous system. Astroglia can support waves of increased intracellular calcium concentration that propagate through the cell, which may provide one mechanism for signaling and communication in glial cells.

MAJOR FINDINGS: The calcium waves are generated by the release and subsequent uptake of calcium by the endoplasmic reticulum (ER). If the cell is modeled as having a heterogeneous distribution of ER, then the response of the cell to agonists is to induce oscillations of calcium with different frequencies at different locations within the cell. These oscillations are coupled by diffusion of calcium (or diffusion of some second messenger, such as IP3). The system can be modeled to a first approximation as a set of weakly coupled, nonlinear oscillators. This model explains some of the phenomena observed experimentally, such as wave collision and annihilation, and provides an explanation for the "calcium fingerprint" hypothesis, which says that each cell has its own unique response to agonists.

PROPOSED COURSE: A model will be developed for calcium wave propagation in glial cells. The kinetics of calcium release and uptake will be described using various published models. The significance on inhomogeneities on wave propagation will be examined. Results will be compared to experimental data and will guide the design of new experiments. A technique is also being developed that allows the ER membrane current to be extracted from the calcium measurements.

PUBLICATION: Roth BJ, Yagodra SV, Holtzclaw L, Russel JT. A mathematical model of agonist-induced propagation of calcium waves in astrocytes. Cell Calcium 1995;17:53-64.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10418-03 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transport of Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cynthia Sung, Ph.D.	Senior Staff Fellow	CHES, BEIP, NCRR
Robert L. Dedrick, Ph.D.	Chief	CHES, BEIP, NCRR
Wanda R. Puckett, Ph.D.	NRC Fellow	CHES, BEIP, NCRR

COOPERATING UNITS *(if any)*
SNB, NINDS (R. J. Youle, V. Vasandani); LMP, NCI (J. N. Weinstein); NM, CC (R. D. Neumann); ALZA Corp. (W. W. van Osdol)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

CHECK APPROPRIATE BOX(ES)
(a) Human subjects X (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An understanding of the factors that govern *in vivo* transport of macromolecules such as ribonculeases, protein toxins, immunotoxins, streptavidin, and monoclonal antibodies (MAbs) will facilitate development of strategies for increasing delivery of these kinds of agents to targeted tissues. Experimental studies, supported by mathematical models, are being conducted in tumor- and nontumor-bearing animals. Measurements of influx and efflux rates and binding parameters of these compounds in various tissues have been obtained. Quantitative autoradiography has been used to characterize the spatial distribution of these compounds in solid tumors. A two-step protocol was investigated that involved the combined use of a modified MAb and a rapidly diffusible substance that binds to the Mab. Spatially distributed pharmacokinetic models were developed to simulate the experimental characteristics of such protocols in order to explore the effects of parameters such as dose, binding affinity, interval between injection of the first and second components, and antigen internalization on outcomes such as total radionuclide uptake, spatial heterogeneity, and tumor:nontumor ratios. Simulations permitted comparison of protocols involving directly radiolabeled MAb with those utilizing streptavidin- or biotin-conjugated MAb. Similar models were applied to the study of the effect of receptor modulation on penetration of protein toxins in solid tumors. A flow chamber was developed to examine the effects of membrane permeability, dose, and dose schedule on the cytotoxicity of protein toxins.

RELEVANT BEIP PROJECTS: 86-128, 89-114, 91-137, and 92-122.

OBJECTIVES: To quantitatively characterize the transport properties of macromolecules by means of experimental and theoretical models, for the purpose of obtaining a clearer understanding of the factors that influence the distribution of these compounds in target and nontarget organs.

METHODS EMPLOYED: (1) Radiolabeled proteins are injected into tumor- and nontumor-bearing mice. The rate of uptake in the tumor and in normal organs is measured at various times after injection. The data are interpreted using a physiological, compartmental pharmacokinetic model. This allows determination of tumor and normal tissue capillary permeabilities of the proteins, lymphatic loss, and binding parameters. Quantitative autoradiography is used to assess the spatial distribution of the radiolabeled protein in a solid tumor following intravenous administration. For the ribonucleases, genetically engineered variants are being studied in order to determine which amino acids are responsible for high retention in the kidneys.

(2) Equations for diffusion, binding, and internalization were employed to describe two-step imaging or treatment protocols. These protocols involve injection of a modified MAb capable of binding both to tumor-associated antigen and to a second component that carries a radionuclide or drug. The second component is injected subsequently, after the MAb has distributed throughout the target tumors and been largely cleared from the plasma and normal tissues. The theoretical model includes the plasma kinetics, transcapillary transport into normal tissue interstitium surrounding the nodule, binding of MAb to antigen, binding of the second component to the MAb, diffusion into a prevascular tumor nodule, internalization of MAb-antigen complexes, and lymphatic outflow from the tissue space. Many of the model parameters were obtained or estimated from published reports or from our own experimental data. We examined two protocols. In the first protocol, the first component is a MAb conjugated to streptavidin and the second component is radiolabeled biotin; in the second protocol, the first component is a biotinylated MAb and the second component is radiolabeled streptavidin. Theoretical simulations of the second protocol are consistent with experimental results obtained from metastatic lung tumors in the guinea pig. Simulations of both protocols, under comparable doses and time between injection of first and second components, suggest that the protocol utilizing a MAb conjugated with streptavidin and radiolabeled biotin is more advantageous pharmacokinetically. However, rapid internalization of antibody-antigen complexes can greatly reduce the advantage over direct MAb targeting and can produce heterogeneous distribution of the second component.

(3) A methodology similar to that used in (2) was employed to simulate the tumor penetration of intravenously administered targeted toxins that differ in molecular weight and mode of cellular processing. Molecular weight is correlated with plasma

clearance, capillary permeability, and interstitial diffusivity. Two paradigms of receptor modulation were considered. In one, the receptor is constitutively expressed; in the other, the receptor is downregulated when exposed to its ligand. Simulations are assisting in the identification of toxin characteristics and of dose schedules that will improve the distribution of a targeted toxin in a solid tumor.

(4) A flow chamber was developed to examine the effects of membrane permeability, dose, and dose schedule on the cytotoxicity of protein toxins. The chamber is divided into two compartments that are separated by a semipermeable membrane. Each compartment contains cells that are sensitive to the toxin. A mathematical model of the flow chamber was developed to aid in designing the device and in specifying optimal operating conditions.

SIGNIFICANCE: The theoretical models permit the testing of modifications of the experimental protocols to anticipate those that will have the greatest impact. Hence, utilization of experimental animals is reduced and a more rational basis for optimization of the procedures is obtained.

PROPOSED COURSE: To continue simulations with spatially distributed models in order to examine effects of molecular weight and receptor modulation. To couple these models with a physiological pharmacokinetic model for distribution of proteins, thereby enabling extrapolation to humans. To measure tumor penetration of small-molecular-weight toxins and genetically engineered variants in order to test model predictions.

PUBLICATIONS: Sung C, van Osdol WW. Pharmacokinetic comparison of direct antibody targeting and pretargeting protocols based on streptavidin-biotin binding. J Nucl Med 1995;36:867-76.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10419-03 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Aluminum in Alzheimer's Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Carol R. Swyt, M.S. Physical Scientist OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
LN, NIA (Q. Smith, D. Brady); CAC, NIST (G. Gillen)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
X (b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of aluminum in the etiology of Alzheimer's disease remains controversial because of the contradictory results reported by the many laboratories in the field. Many of the laboratories relied on fixatives and stains to facilitate the unambiguous identification of plaques and neurofibrillary tangles (NFTs), the indicators of the disease. However, the chemical processes of fixing and staining compromise the distributions and concentrations of aluminum and other elements of interest. Measurements on unfixed, unstained sections in which elemental distributions are assumed to be preserved are questioned because the identification of the structures being measured is difficult. A methodology has been developed that permits the unambiguous identification of plaques and NFTs in unfixed, unstained sections, that does not alter the elemental distributions, and that provides detectability and rapid imaging of the aluminum distribution at concentrations in the ppm range. This methodology uses thioflavin-stained sections for observation by fluorescent LM, and freeze-dried cryosections for observation by backscattered-electron imaging (BSI) and for quantitative measurement by electron-induced X-ray microanalysis and SIMS. No aluminum above the normal background concentration has been observed in tangled neurons in sections prepared by this method. However, a marked increase in sulphur relative to phosphorus has been observed in NFTs, and of Fe in plaques.

METHODS EMPLOYED: Specimens are obtained from Alzheimer's brain hemispheres frozen at autopsy and maintained at -80°C. Sections are cut on a cryomicrotome; one section from a series is air-dried, treated with a modified thioflavin stain, and photographed in the LM to document NFT and plaque-rich regions. An adjacent section is examined using back-scattered electron imaging (BSI) in the JEOL JXA-8600 electron probe microanalyzer (EPMA). Intensity in the BSI images increases with density and average atomic number. Previous work that compared BSI images to LM images of the same sections post-stained with thioflavin has verified that correct identification of structures may be made in BSI. The elemental composition of these structures in the sections is obtained in the EPMA by energy-dispersive X-ray spectrometry (EDS). Higher sensitivity (20 ppm weight fraction for Al) wavelength-dispersive X-ray spectrometry (WDS) is used to measure Al. Because final cryosection thickness in the 5-20 μm regime is difficult to control and because the optimum operating voltage for good contrast in BSI is 20-30 kV, there is an unknown substrate contribution to the continuum. Absolute quantitation by conventional peak to background methods is therefore very inaccurate. The spectrum generation functions and Simplex peak fitting algorithm of the Desk Top Spectrum Analyzer and X-Ray Database program (DTSA) are used for accurate determination of all but trace element concentrations. This is accomplished by generation of spectra with peak areas equal to those measured in real spectra by the DTSA.

SIGNIFICANCE: The contradictory results of the many measurements of aluminum in Alzheimer's brain specimens leave the role of this element in the disease process in question. It is uncertain if the extreme variation in results has been due to the preparation procedures or to real differences among the samples measured. A methodology that enables the identification of plaques and NFTs, that maintains the elemental distributions, and that permits measurement of the concentration and distribution of aluminum at the ppm or ppb level would contribute to eliminating that uncertainty and to determining the significance of aluminum in the Alzheimer's disease process.

PROPOSED COURSE: Specimen cryosectioning and freeze-drying will be improved to minimize ice-crystal damage and to facilitate identification of neurons and plaques by BSI. Samples will be collected from specimens acquired and rapid-frozen at autopsy, and exhibiting very well-defined characteristics of AD. EDS spectra will be collected from 'matrix', NFTs, plaques and the endothelium of venules,etc from these specimens and controls.

PUBLICATIONS: Smith QR, Deng QS, Brady DR, Swyt CR, Nguyen J, Gillen JG. Aluminum localization and analysis in stained and unstained human brain tissue using X-ray microanalysis and SIMS. In: Gitelman H, ed. Proceedings of the Third International Conference on Aluminum in Health. Miami: NIA, 1994 (in press).

Gillen JG, Nguyen J, Swyt CR, Deng QS, Brady DR, Smith QR. Methods for localization and imaging of trace element distributions in human brain neurons using SIMS. In: Benninghoven A, ed. Secondary ion mass spectrometry (SIMS). Chinchester: John Wiley and Sons, 1994;585-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10420-03 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thymidylate Synthase Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Paul F. Morrison, Ph.D. Physical Scientist CHES, BEIP, NCRR

COOPERATING UNITS *(if any)*
NCI-NMOB (C. Allegra, E. Chu, D. Voeller)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biosynthesis of thymidylate synthase (TS) has recently been found to be down-regulated by binding of the protein to its own mRNA, and this regulation is involved in the development by cancer cells of resistance to several antimetabolites targeted at this enzyme. Because both TS-mRNA binding to TS and TS-enzyme activity were observed to increase similarly with increasing mercaptoethanol (ME) concentration, we tested whether these affinity and activity increases were consistent with either the hypothesis of control by a single common redox site or the hypothesis of independent control of binding and activity by two separate sites. Thus, data sets describing both the binding of TS to TS-mRNA and the activity of TS (no mRNA present) with added ME were fit separately to models accounting for binding to two known mRNA sites, for thymidylate production, and for appropriate choice of putative redox control. Likelihood ratio testing for either redox model revealed that the redox parameter vector is very significantly different when estimated from the binding or the activity data, thus leading to the rejection of both hypotheses. Hence, redox regulation of TS synthesis appears to be complex, involving coupled interactions between multiple sites. In addition, some structural investigation of the TS-mRNA binding region was begun. Melting points of two possible 35-mer stem-loop structures, identified previously, were examined for UV absorbance and found to be consistent with predicted G-C-rich stems. Furthermore, because the stem-loop structures differ in having a single adenine either free or base-paired, 1D-NMR was performed to seek evidence for far down-field-shifted imidazole hydrogens of U-paired adenine. Shifting was less than 13 ppm, thus indicating the need for 2D-NMR.

RELEVANT BEIP PROJECTS: 93-104, Thymidylate Synthase Regulation.

OBJECTIVES: To physically characterize and quantify the regulation of thymidylate synthase production controlled by the binding of that protein to its own messenger. To explore this regulation in order to identify agents or protocols useful in circumventing some of the drug resistance associated with up-regulation of this system.

METHODS EMPLOYED: We wished to test whether the increases in binding affinity and enzyme activity that occurred with increasing mercaptoethanol (ME) concentration were consistent with control by a single common redox site or with control of binding and activity by two separate and independent sites, most likely involving cysteine groups on the surface of the protein. Accordingly, redox models were formulated that accounted for binding to two known mRNA sites, for thymidylate production (using an ordered reaction mechanism with initial binding by dUMP), and for either one or two putative ME reactions, as well as relevant mass balances and stoichiometries. The putative redox reaction(s) between enzymatically active and inactive TS, 2-mercaptoethanol, and 2-hydroxyethyldisulfide was(were) described by equilibrium constant K*, while the binding between active TS and the two known mRNA binding regions was described by equilibrium constants K1 and K2. The model expressions were fit separately to binding and enzyme activity data as a function of ME concentration with local variance weighting to determine mean parameter values and their variance-covariance matrix. Likelihood ratio testing was used to reject or accept the redox-control hypotheses. Experimental determination of the concentrations of TS/mRNA^{TS} complexes involved binding of ^{14}C-labelled messenger to TS, followed by digestion of the unbound mRNA segments by T1 nuclease and subsequent resolution of the complexes on polyacrylamide gels with quantitation by autoradiography. Melting-point experiments were conducted by observing UV absorbance at 260nm in RNase-free solutions over the range of 14° to 95°C. mRNA secondary structure predictions are from the FOLD and SUBOPTIMAL routines.

MAJOR FINDINGS: The major finding from likelihood ratio testing was that the parameter vector common to the activity and binding fits is very significantly different when estimated separately from the binding or the activity data, thus leading to the rejection of both the common redox site control hypothesis and the two independent redox sites control hypothesis. The major structural findings are that (a) several mRNA secondary structures are closely grouped in inergy, and that (b) one cannot discriminate between the most likely structures simply by identification of a unique A-U pairing in one structure by 1D-NMR demonstration of a far down-field-shifted imidazole hydrogen of adenine.

SIGNIFICANCE: The observation that both binding and enzyme activity are under reversible redox control in TS parallels similar observations in the bacteriophage-coat protein, aminoacyl-tRNA synthetase, and in iron-responsive element-binding protein systems, suggesting that further study of TS and its messenger may reveal details of a general mechanism of protein synthesis regulation, as well as potential drug-targeting sites. Our modeling indicates that the coupling between these multiple sites and affinity/activity is likely to be intricate. In agreement with our findings, recent NMOB experimental determinations of TS mRNA binding to various TS mutants, including those with altered cysteine residues, are in accordance with intricate multiple site control, since a wide range of affinities has been measured with no evidence for dominant control by a single site. The significance of the structural findings is that extensive 2D-NMR is required for characterization of the mRNA stem-loop structure.

PROPOSED COURSE: The binding between TS and its messenger is thought to occur between a surface site on TS and a stem-loop structure of $mRNA^{TS}$. At present, the mRNA stem-loop configuration, a potential drug target, is not known. Thus, we plan further free-energy calculations to determine the liklihood of multiple stem-loop forms coexisting at 37°C, as well as collaborative two-dimensional NMR structure experiments to determine if one structure dominates at this temperature. Very recent work at NMOB has also identified a 28 amino acid peptide that binds very strongly to the stem-loop portion of TS-mRNA. Aspects of the binding and drug delivery of this agent are to be considered.

PUBLICATION: Chu E, Voeller DM, Morrison PF, Jones KL, Maley GF, Maley F, Allegra CJ. The effect of reducing reagents on binding of thymidylate synthase protein to thymidylate synthase messenger RNA. J Biol Chem 1994;269:20289-93.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10422-03 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biomedical Applications of Optical Low-Coherence Reflectometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert F. Bonner, Ph.D.	Biophysicist	EEES, BEIP, NCRR
Michael Yadlowsky, Ph.D.	NRC Fellow	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

IR CE, NHLBI (A. Knüttel); IR LCB, NHLBI (J. Knutson); PSL, DCRT (A. Gandjbakhche); Elec. Eng. Dept., Hong Kong Univ. Sci. & Tech. (J. Schmitt)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.9	0.1

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Optical-coherence reflectometry (OCDR) was first introduced as a method for high-resolution probing of reflections from components of fiber-optic systems. We have been exploring the applications of this technique to imaging and to characterization of biological tissues. Using prototype instruments built in our laboratory, we have succeeded in imaging subsurface structures in living skin and other tissues. To help understand the limits of contrast and resolution that can be attained with this new type of microscopy, mathematical models of the propagation of partially coherent light in dense tissue have been constructed and tested in experiments on tissues and tissue phantoms. Our results show that without staining or modifying a tissue in any way, structures embedded 1 mm to 2 mm in skin tissue can be imaged with a resolution of about 10 µm.

OBJECTIVES: To develop a new imaging modality that permits noninvasive probing of subsurface structures in living tissue. We envision an inexpensive, handheld scanner that could be used to perform "optical biopsies" of skin lesions. Coupled to an endoscope, the scanner could have a variety of applications in minimally invasive surgery.

METHODS EMPLOYED: An optical-coherence reflectometer is built around an interferometer illuminated by a continuous-wave broadband light source. The mirror in one of the arms of the interferometer is replaced by the sample, and the mirror in the other (reference) arm is translated to vary the optical-path difference. Fringes are produced only when the optical path of the light reflected from the sample and that reflected from the reference mirror differ by less than the coherence length of the light source. When light-emitting diodes (which emit light with a short temporal coherence) are employed as sources, pathlength differences as small as a few microns can be resolved. By recording the fringe magnitude versus the displacement of the reference mirror, we can obtain a profile of the optical power backscattered by the sample over a range of optical depths.

Several low-coherence reflectometry systems based on these principles have been constructed in our laboratory and have been used to measure backscattered signals from living tissues and scattering models with tissue-like properties. Our latest system, based on an interferometer built with single-mode optical fibers, operates at a wavelength of 1300 nm. It combines features of confocal and optical-coherence microscopes. To analyze the backscattered intensity-versus-depth profiles obtained from the samples, we have developed first-order scattering models that have recently been extended to include effects of multiple forward scattering.

MAJOR FINDINGS: Knowledge gained over the last year through modeling and experimentation has enabled us to improve greatly the quality of the images obtained with the prototype microscope from tissue. In living skin, details of the organization of the cells in the epidermis and contours of the dermo-epidermal junction can now be visualized readily. In excised arterial specimens, the banding of the elastin fibers and the transition between the media and adventitia can be seen. In order to improve image quality further, we need to better understand the contrast mechanisms underlying the variations in the optical properties imaged with the microscope, as well as exploiting other contrast-enhancement methods that have not yet been tried.

SIGNIFICANCE: Imaging and spectroscopy using low-coherence interferometry may potentially permit noninvasive diagnosis or characterization of superficial lesions in any tissue accessible from the surface of the body. Assessment of retinal damage, early detection of skin melanomas, and measurement of burn depth are a few of the specific applications that have been suggested. In addition, we can imagine a variety of applications in biomedical research, such as quantifying the extent of atherosclerosis and visualizing the effects of stress on the growth of cartilage.

PROPOSED COURSE: One major goal of our future work will be to understand the contrast mechanisms underlying optical-coherence imaging. We plan to use a combined experimental and theoretical approach to reach this goal. Having already worked out most of the fundamental technical problems of the microscope in its basic configuration, we intend to incorporate more advanced features into future versions. These features may include multiple-wavelength sources for differential-absorption imaging, multiple detectors for spatial-frequency discrimination, and polarization modulation for polarization-contrast imaging. Once the performance and reliability of the optical-coherence microscope have been proven, collaborative clinical studies will begin.

PUBLICATIONS: Schmitt JM, Knüttel A, Yadlowsky M. Confocal microscopy in turbid media. J Opt Soc Am A 1994;11:2226-35.

Schmitt JM, Knüttel A, Yadlowsky M, Eckhaus MA. Optical-coherence tomography of a dense tissue: statistics of attenuation and backscattering. Phys Med Biol 1994;39:1705-20.

Yadlowsky MJ, Schmitt JM, Bonner RF. Coherence degradation in turbid materials probed by optical-coherence reflectometry. Appl Optics 1995 (in press).

Schmitt JM, Yadlowsky MJ, Bonner RF. Subsurface imaging of skin with optical coherence microscopy. Dermatology (in press).

Yadlowsky MJ, Schmitt JM, Bonner RF. Contrast and resolution in the optical-coherence microscopy of dense biological tissue. Proc SPIE 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10424-03 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NMR Center Workstations and NMR Center Internetwork (SPINet)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR
Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NMR Center maintains a collection of UNIX-based workstations for use by the NMR Center user community. The demand for off-line processing is still increasing significantly. In the past year, SPARC10 workstations have been added, along with an upgrade of the Sun file servers. The system is now connected with MRIPS, through which disk space can be purchased as needed by the users. Additional workstation upgrades or new workstations will be purchased as the need arises. These workstations are connected to the NMR Center network, which provides access to all the NMR instruments in the Center, as well as to the NIH backbone.

Another responsibility is the design, installation, testing, performance monitoring, and maintenance of the NMR Center network (SPINet). The NMR Center contains an internetwork consisting of thick-ethernet, thin-ethernet, and local-talk physical media. This internetwork uses the TCP/IP and Appletalk protocol suites. A variety of bridging and interconnection devices are used to interconnect the various physical and logical networks.

As the NMR Center grows, the network will continue to be adapted.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10425-03 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal MRI Systems Maintenance, Protocol Development, Operator Training

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alan W. Olson, M.S.	Electronics Engineer	NMR, BEIP, NCRR
Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR

COOPERATING UNITS *(if any)*

Alan McLaughlin (NIMH); Barnett (NINDS)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

In Vivo NMR Research Center

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.8	PROFESSIONAL: 0.7	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NMR Center contains two animal MRI instruments: a 2.0-Tesla, 45-cm-bore instrument and a 4.7-Tesla, 40-cm-bore instrument. The systems are kept at a state-of-the-art level by upgrades of principal components. The principal investigator performs periodic performance checks, routine component changes, and cryogen ordering and filling as needed for operation of the systems, and assists system operators as needed with protocol development and continuing training. Investigators from the cooperating units occasionally assist the principal investigator (Dr. Barnett helps with the 2.0-T instrument, and Dr. McLaughlin helps with the 4.7-T instrument).

During this period, a 3-cm-bore, ultrafast gradient set was installed for microimaging, in addition to a 15-cm-bore, shielded gradient set. We also anticipate upgrading the console of one of the instruments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10426-03 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Apple Macintosh Computer Administration at the NIH *In Vivo* NMR Research Center

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR
Denise Sobering, B.A.	Secretary	NMR, BEIP, NCRR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

In Vivo NMR Research Center

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The *In Vivo* NMR Research Center maintains a collection of Macintosh computers and peripherals that are used in the daily operation of the Center, as well as for various research tasks. The demand for the use of Macintosh computers for off-line processing of data from all the Center's magnets is increasing as the distinction fades between workstations and high-end Macs. The Center's Macintosh computers are also in high demand for the preparation of images to be submitted to journals and societies, because image software and peripherals are relatively cheap and user-friendly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10427-03 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of *In Vivo* Proton NMR Spectroscopy and Spectroscopic Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR
Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR

COOPERATING UNITS *(if any)*
LDRR, OD (J. Frank, J. Duyn); CBDB, NIMH (D. Weinberger); Johns Hopkins University (P. van Zijl); Pittsburgh NMR (J. Gillen); University of Utah (P. Tang)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is the noninvasive evaluation of regional biochemistry, the human brain in particular. Programs developed in the NMR Center are now considered the state of the art. Software has been requested and installed at Massachusetts General Hospital, Boston; McLean Hospital, Boston; the University of Madrid, Spain; the University of Sydney, Australia; the University of Utah; and the University of Kyoto, Japan. Applications are ongoing in the fields of acute stroke, adrenoleukodystrophy, glioma and lymphoma tumors, and schizophrenia.

OBJECTIVES: To develop an improved multi-slice, multi-spin-echo proton spectroscopic imaging method for human brain.

METHODS EMPLOYED: During the last year, emphasis has been put on the development of fast proton spectroscopic imaging programs and on automatic processing. Measurements were performed with a standard 1.5-Tesla clinical scanner on healthy volunteers and on a patient with high-grade CNS lymphoma.

MAJOR FINDINGS: The technique allows the reconstruction of lactate images, along with choline plus creatine, N-acetylaspartate, and lipid images from one single data set processed in three separate ways. The discrimination between resonances of lipid protons and lactate methyl protons is based on homonuclear spin-spin coupling. The reliability of the separation of the lipid and lactate contribution depends on the T_2 of the lipid resonances.

SIGNIFICANCE: We have demonstrated the ability to obtain high-quality metabolite maps within 11 minutes.

PROPOSED COURSE: We expect to continue these efforts, particularly in the field of processing. We will modify the existing routines to the new systems with fast gradients. We also plan to investigate other approaches to fat suppression.

PUBLICATIONS: Kruse B, Barker PB, van Zijl PCM, Duyn JH, Moonen CTW, Moser HW. Multislice proton MR spectroscopic imaging as an aid to phenotype assignment and prognosis in X-linked adrenoleukodystrophy. Ann Neurol 1994;36:595-608.

Duyn JH, Frank JA, and Moonen CTW. Incorporation of lactate measurement in multi-spin-echo proton spectroscopic imaging. Magn Reson Med 1995;33:101-7.

Tedeschi G, Bertolino A, Righini A, Campbell G, Raman R, Duyn JH, Moonen CTW, Alger JR, DiChiro G. Brain signal distribution pattern of metabolite signal intensities in young adults by proton magnetic resonance spectroscopic imaging. Neurology (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10428-03 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of the Use of Field Gradients in High-Resolution NMR Spectroscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR
Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR

COOPERATING UNITS *(if any)*

Johns Hopkins University (P. van Zijl)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

In Vivo NMR Research Center

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects
- (a1) Minors
- (a2) Interviews

(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project, the plan is to speed up multidimensional spectroscopy and to acquire new information with the help of self-shielded, high-performance field gradients.

PROPOSED COURSE: With the increasing attention on functional imaging, we anticipate that this collaborative research project will be phased out.

PUBLICATIONS: Moonen CTW, van Zijl PCM. Water suppression in proton spectroscopy of human and animals. Encyclopedia of Nuclear Magnetic Resonance (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10429-03 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Functional MRI Approaches

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR
Guoying Liu, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR
Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR
Peter van Gelderen, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR
Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR

LDRR, OD (J. Frank, J. Duyn); CBDB. NIMH (D. Weinberger, N. Ramsey); Barker (Henry Ford, Detroit); Cacace (Albany)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

In Vivo NMR Research Center

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to acquire functional data in addition to the high-quality anatomical data currently obtained with MRI. The following functions are being studied: flow, perfusion, diffusion, oxygen consumption, and metabolism. Aspects under study are NMR pulse sequences, image processing, and relation to PET.

METHODS EMPLOYED: Of particular importance during this period were the developments in perfusion imaging and in imaging of brain activity. This approach uses large magnetic susceptibility weighting based on the echo-shifting principle combined with multiple gradient echoes per excitation.

MAJOR FINDINGS: Motor stimulation, induced by self-paced finger tapping, reliably produced significant signal increase in the hand region of the contralateral primary motor cortex in every subject tested.

SIGNIFICANCE: An efficient 3-dimensional fMRI method is presented for imaging brain activity on conventional, widely available, 1.5-T scanners, without additional hardware. We anticipate that this field will remain at the focus of the technological development program of the NMR Center, particularly following the arrival of faster, more powerful gradients.

PROPOSED COURSE: Areas of technical innovation will be directed toward the use of advanced radiofrequency spoiling techniques, as well as toward the use of navigator echoes to improve signal stabilities. We also plan to incorporate advanced 3D registration routines developed in Dr. Unser's image processing group. In addition, the research will focus on the relation between the NMR signal and local physiological changes upon its activation.

PUBLICATIONS: van Gelderen P, Ramsey NF, Liu G, Duyn JH, Frank JA, Weinberger DR, Moonen CTW. Three-dimensional functional MRI of human brain on a clinical 1.5-T scanner. Proc Natl Acad Sci USA (in press).

van Gelderen P, Duyn JH, Moonen CTW. Susceptibility insensitive single-shot MRI combining BURST and multiple spin echoes. Magn Reson Med 1995;33:422-39.

Mattay VS, Weinberger DR, Barrios FA, Sobering GS, Duyn JH, Kotrla KJ, Sexton RH, Moonen CTW, Frank JA. Brain mapping using functional MRI: a direct comparison of gradient echo based dynamic contrast and "BOLD" methods. Radiology 1995;194:687-91.

Moonen CTW. Functional MRI (Editorial). Biol Psychiatry 1995;37:141-3.

van Gelderen P, Duyn JH, Moonen CTW. Analytical solution for phase modulation in BURST imaging with optimum sensitivity. J Magn Reson B 1995;107:78-82.

van Gelderen P, Duyn JH, Liu G, Moonen CTW. Optimal T2* weighting for BOLD-type functional MRI of human brain. Indian Academy of Sciences, Chem Sci 1994;106:1617-24.

Duyn JH, van Gelderen P, Barker P, Frank JA, Moonen CTW. 3D bolus-tracking with frequency-shifted BURST MRI. J Comput Assist Tomogr 1994;18:680-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10430-03 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional MR Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ronald L. Levin, Sc.D. Biomedical Engineer MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
NIMH (J. Haxby)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the past several years, it has become apparent that magnetic resonance (MR) can provide information other than of a purely anatomical or diagnostic nature. MR has been shown to be sensitive to physiological and pathological processes, particularly in the brain. These so-called "functional" MR techniques may one day rival existing PET (Positron Emission Tomography) techniques as the methods of choice for the noninvasive visualization and analysis of neural activity. As such, NIH is starting a large multi-ICD effort in the area of functional MR research. BEIP has been asked to play a supporting role in this effort.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To facilitate the use of magnetic resonance imaging and spectroscopy for the functional visualization of brain activity.

PROPOSED COURSE: (1) To develop MR-compatible test equipment similar to that already available for PET-based functional research, for the stimulation of visual, auditory, and human motor-control activity under well-controlled conditions, using echo-planar functional MR scanning. (2) To use the NIH's new MEDx image-processing package for the visualization and analysis of functional MR data.

RECENT ACCOMPLISHMENTS: During the past year, several additional routines to facilitate the visualization and analysis of fMRI studies have been added to MEDx. Also, a new specialized MR-compatible goggle system has been procured to facilitate fMRI experiments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10431-03 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of *In Vivo* NMR Diffusion Spectroscopy and Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR
Peter van Gelderen, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR
Guoying Liu, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR
Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR

COOPERATING UNITS *(if any)*

University of Delft, The Netherlands (T. Mehlkopf); Johns Hopkins University (P. van Zijl)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

In Vivo NMR Research Center

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.6	0.1

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is the study of (restricted) mobility of metabolites and water for a clarification of local physical chemical parameters, such as viscosity, membrane transport, and membrane permeability.

MAJOR FINDINGS: A major application of *in vivo* NMR diffusion spectroscopy and imaging is the study of the diffusion of water in acute stroke. It is now well established that the diffusion coefficient of water decreases significantly in acute stroke in areas of cytotoxic edema. Its detection opens the way for pharmaceutical intervention.

SIGNIFICANCE: NMR spectroscopy offers the opportunity to study molecular diffusion in living systems noninvasively. The data from such studies can be used to determine the mobility of compounds in cells and tissues, to estimate the size of compartments, and to monitor transport and exchange.

PROPOSED COURSE: During the next year, we will focus on the development of advanced methods to measure diffusion on clinical whole-body NMR instruments.

PUBLICATIONS: Van Zijl PCM, Davis D, and Moonen CTW. Diffusion spectroscopy in living systems (review). In: Gillies R, ed. MR in physiology and medicine. San Diego: Academic Press, 1994; 185-96.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10445-02 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Subcellular Ion Distributions in *Acanthamoeba castellanii*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR
Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

LCB, NHLBI (B. Bowers)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues X (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The contractile vacuole (CV) is an osmoregulatory organelle found in amoebae that live in hypotonic environments. Calmodulin, alkaline phosphatase, and a proton pump are known to be associated with the CV and its spongiome of tubules. However, the ion gradients generated across the CV membrane and their role in the water-pumping mechanism remain unknown. An improved understanding of CV water regulation may be relevant to the physiology of other systems, such as the vertebrate kidney. The techniques of rapid freezing, cryosectioning, scanning transmission electron microscopy, x-ray spectroscopy, and electron energy-loss spectroscopy have been adapted to measure CV ion concentrations in cryosections of rapidly frozen *Acanthamoeba castellanii*. Low-dose dark-field STEM has provided an estimation of the ion concentrations in dilute subcellular compartments for the first time. For cells in standard growth medium, preliminary results indicate a [Na+]:[K+] ratio of ~6:1; low [Cl-], suggesting HCO_3^- as the major anion; and a total osmolality of ~21 mosmoles. This new approach allows us to explore how external hypotonic and hypertonic ionic environments affect contracile vacuole ion concentrations.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To measure, by means of analytical electron microscopy, ion concentrations in the contractile vacuole of *Acanthamoeba castellanii* grown in a variety of media.

METHODS EMPLOYED: *Acanthamoeba* was incubated with 5% PVP (polyvinylpyrrolidone) and was centrifuged at low speed to obtain a high-density suspension of cells. This suspension was frozen in liquid ethane, cryosectioned to a thickness of 100 nanometers, and transferred to carbon-coated EM grids. Specimens were cryotransferred at -180°C into a field-emission STEM, and parallel EELS spectra were acquired at low dose to determine the mass thickness of the hydrated section. The specimen was then dehydrated by slowly warming to -100°C, and quantitative dark-field images were recorded, also at low dose; this provided the mass of the salts deposited from the dilute compartment relative to the mass of the underlying support film. Finally, in order to determine the ratios of ions, energy-dispersive x-ray spectra were recorded at high electron dose from the regions of the dried salts.

MAJOR FINDINGS: Use of analytical electron microscopy to determine ionic concentrations in very dilute submicrometer-sized cellular compartments has not been possible previously. This is because the standard Hall Method for x-ray microanalysis of freeze-dried cryosections requires an organic matrix to calculate the dry mass fractions of ions, from which the molar ion concentrations can be estimated if a value for the water content is assumed. X-ray microanalysis of hydrated cryosections is also precluded because of the extreme susceptibility of vitrified water or ice to radiation damage in the electron beam. By using a combination of dark-field scanning transmission electron microscopy (STEM), electron energy-loss spectroscopy (EELS), and energy-dispersive x-ray spectroscopy (EDXS), we were able to overcome this limitation.

SIGNIFICANCE: The combination of STEM, EELS, and EDXS offers the possibility of determining ion concentrations in dilute cellular compartments quantitatively for the first time.

PROPOSED COURSE: To obtain further compositional measurements of contractile vacuoles and other compartments in amoebae grown in a variety of media.

PUBLICATIONS: Shi SL, Sun SQ, Bowers B, Leapman RD. Measurement of ion concentrations in very dilute cellular compartments by analytical electron microscopy. In: Proceedings of the 29th annual MAS meeting. 1995 (in press).

Bowers B, Shi SL, Sun SQ, Leapman RD. Ion composition of the contractile vacuole of *Acanthamoeba castellanii* as determined by x-ray microanalysis. In: Proceedings of the 53rd annual meeting of the Microscopy Society of America. 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10446-02 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Nitric Oxide Measurement System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alec Eidsath, Ph.D.	Chemical Engineer	MES, BEIP, NCRR
Stephen B. Leighton, Sc.D.	Mechanical Engineer	MES, BEIP, NCRR

COOPERATING UNITS *(if any)*

CCM, CC (W. Vandivier)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Mechanical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects
_(a1) Minors
(a2) Interviews

(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Endogenous nitric oxide (NO) has been reported in the exhaled air of humans and other species. It is of interest to measure the concentration of NO in real time in the exhalations of patients with a variety of conditions, including those in intensive care settings. We are developing a system to make these measurements continuously at the ppb level.

OBJECTIVES: The measurement system will be portable and compatible with mechanical respirators. The system will use a commercial ozone-type NO monitor and a commercial flow meter for instantaneous respiratory flow-rate monitoring. Data will be digitized and analyzed under the control of a "virtual instrument" written in the LabView programming language. A custom calibration system based on standard gas mixtures will also be included.

METHODS EMPLOYED: The existing commercial NO monitor (Sievers) will be run in a continuous mode, drawing less than one percent of the exhaled air from the mouthpiece of the respirator. At nearly the same location in the external airway, a commercial flow meter transducer (Bicore) will measure instantaneous flow rate, as well as airway and esophageal pressures. At the same time, the EKG, arterial pressure, oxygen saturation, and capnogram will be digitized and saved. There will be eight input analog lines and a serial port read of the Bicore containing the two pressures and the two flow rates. After interpolating the data to put the flow rates and the NO concentration at the same sampling interval, the two signals can then be multiplied, thus deriving the absolute amount of NO as a function of time. Other algorithms such as amount per breath can be implemented as particular experiments require. Calibration is achieved with three standard NO mixtures in various proportions, monitored by variable-area flow meters.

MAJOR FINDINGS: The system has been built and tested. Calibration shows a linear response as low as 5 ppb. The sampling system is adequate. The time lag of the NO signal with respect to the flow signal, due to the finite travel time of the sample from the mouthpiece to the analyzer, can be subtracted in the PC to align all the signals. The NO concentration can then be convolved with flow rate to provide a curve of absolute NO production versus time. The half-life of NO is long enough that it does not interfere with the measurement. Analysis of possible gas dispersion during transit shows that this is not a problem. The continuous signal, correlated with the breathing cycle, permits simple monitoring of NO concentration in the inhaled air, as well, allowing simple subtraction of background levels.

SIGNIFICANCE: This system will permit a variety of clinical studies of the significance of NO in disease. Volunteers can be accommodated without the use of a respirator and with no additional work. The continuous nature of the measurement permits analysis of variations within the breathing cycle; such analysis was not possible with previous batch-mode measurements. The user thus has access to end tidal NO and to other derived parameters.

PROPOSED COURSE: Further programming will permit additional algorithms to be implemented as they are required. Other experiments will involve the study of endotoxins and NO donors (e.g., nitroprusside). Septic-shock victims will be studied. The same experimental setup will be used to examine cyclical changes in blood pressure, EKG rate, and pulmonary muscle function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10447-02 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Aluminum Standards for Alzheimer's Disease Research

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Carol R. Swyt, M.S. Physical Scientist OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
LCNSS, NINDS (R. Garruto); LN, NIA (Q. Smith); CAC, NIST (G. Gillen, R. Fletcher)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
X (b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data on the concentrations and distributions of aluminum (Al) in Alzheimer's disease (AD) specimens reported by various laboratories are difficult to compare for several reasons, including different microanalytical techniques, specimen preparation procedures, artifacts, and detection limits. The fundamental information necessary to interpret and to quantitatively compare data (image data in particular) has been lacking in many published results. Sample populations have not been selected to a consistent standard of disease definition. As a result, not only has it been impossible to clarify the role of Al in the etiology of the disease, but it has been impossible even to determine the conditions under which it may have a role. To begin to address the problem of data comparison, two steps have been taken: Al-doped brain homogenates have been investigated as potential multitechnique standards, and a special Issue Group on Aluminum Biological Standards has been organized for the Workshop on Research Issues in Aluminum Toxicity (Vancouver, July 8, 1995) to propose a common standard and acceptable experimental procedures for the various analytical techniques used in AD. The results described here are included in the Issue Group Summary, which proposes Al-doped brain homogenates as the multielement standard.

METHODS EMPLOYED: Normal brain homogenates doped with Al standard solution to give nominal dilutions ranging from 1000 to 1 ppm have been frozen, cryosectioned (3 µm to 20 µm), air-dried and freeze-dried, and analyzed by scanning ion mass spectrometry (SIMS) and electron probe microanalysis (EPMA). Total analytical volume composition of all but trace concentration elements was determined from EDS spectra using the Desktop Spectrum Analyzer and X-Ray Database program. This information was used to quantitate single-line WDS spectra, which give detectibility for Al of about 20 ppm weight fraction.

SIGNIFICANCE: SIMS measurements have shown good agreement with AA values, and linearity over the range of concentrations. However, the better spatial resolution (<1 µm) energy dispersive x-ray spectrometry and wavelength dispersive x-ray spectrometry measurements have revealed two problems, which typify the difficulties of intertechnique comparison of AD results: 1) inhomogeneity of Al distribution at less than 1 µm spatial resolution; and 2) variation in composition of the analytical volume from low to high dilutions, which affects EPMA quantitation. However, with the total volume composition obtained by EDS, accurate quantitation of WDS data is possible; and, with corrections for composition, there is good agreement of averaged WDS values with SIMS and AA. This analytical strategy could be applied to EPMA spectra acquired at the full range of spatial resolutions of the various techniques, and also to resin-embedded homogenates. Sections of embedded material are the preferred specimen for both microproton-induced x-ray emission (micro-PIXE) and laser microprobe mass spectrometry (LMMS). EPMA could thus provide normalization among the techniques, as well as high spatial resolution analysis. The ability to compare results from the various techniques increases the total sample size of Alzheimer's specimens, enables differentiation of possible subsets, and facilitates identification of environmental and other factors that influence the development of the disease.

PROPOSED COURSE: The mixing, freezing, and drying procedures will be modified to improve homogeneity of the aluminum distribution in the homogenates. Both embedded and freeze-dried sections of Al-doped homogenates will be examined by high spatial resolution EPMA to measure the homogeneity of the Al distributions. Embedded sections will be analyzed by LMMS and EPMA, and procedures for rationalizing the results from the different preparations will be developed. A round-robin analysis of homogenates will follow, to assess the analytical procedures followed by the participating laboratories.

PUBLICATIONS: Lovell MA, Ehman WD, Markesbury WR, Melethil S, Swyt CR, Zatta PF. Aluminum biological standards: what are the needs? J Toxicol Environ Health (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10448-02 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Patient Simulator for Hemofiltration Procedures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas Talbot, M.S. Mechanical Engineer ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*
NURS, CC (C. Rosenthal, V. Strider)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A patient simulator was conceived, designed, and fabricated to supplement classroom instruction of critical-care nurses in hemofiltration procedures and enable guided practice opportunities. The simulator consists of a mock circulatory system, complete with venous and arterial reservoirs, venous and arterial lines, and adjustable arterial pressure and venous resistance. The device also includes the hardware necessary to produce clinical alarm states in certain pump-assisted hemofiltration procedures. In addition, the device has proved useful in evaluating the suitability of the support equipment needed to perform renal-replacement therapies in a clinical setting.

PHS 6040 (Rev. 5/92)

PROPOSED COURSE: The device is completed and is now in use in the Medical Intensive Care Unit. No further development is anticipated for this device.

PUBLICATIONS: Talbot TL, Rosenthal CH, Strider VC. Collaborative development of a patient simulator for educating nurses in hemofiltration therapies. Biomed Instrum Technol 1994;28(4):271-81.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10450-02 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effect of Temperature Gradients on Calorimetric Measurements in Fluids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LPB, NIAMS (N. Gershfeld); IR CH, NHLBI (R. Berger)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(a1) Minors

(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Due to the dynamic nature of the scanning process, a temperature gradient exists throughout the fluid samples during scanning calorimeter measurements. As a result, when a scanning calorimeter is used to make heat-capacity measurements, the effect of the lagging temperature gradient in the sample distorts the output when a phase transition occurs. Over the years, several schemes have been developed to correct this distortion; to date, however, no one has attempted to quantitate the temperature gradient in the sample and determine its actual relationship to the measured output. We have developed detailed, two-dimensional RC models of several calorimeter designs, and have evaluated the dynamic temperature gradients in the fluid samples during the measurement process, using a commercial circuit-simulation (SPICE) program. These analyses have both revealed the source of several temperature gradient-induced errors in the measurements, and allowed us to redesign and re-evaluate new calorimeter designs in software before they are constructed, in order to reduce the magnitude of these errors.

PHS 6040 (Rev. 5/92)

OBJECTIVE: To investigate the relationship between the temperature gradients observed in fluid samples being measured in calorimeters, and the measured outputs of the instruments, in two ways: (1) using an RC model of the thermal measurement system, and simulating it in a SPICE program; and (2) investigating the use of several newly developed FES thermal-analysis programs for the PC platform.

SIGNIFICANCE: During dynamic measurements on fluid samples in nonadiabatic calorimeters, the measured output is typically due to a temperature change at the sensor, which is usually not in the sample. Under these conditions, it is important to establish the relationship between the measured output and the thermal event in the sample. In the past, this relationship was usually determined empirically by calibration, using known reactions or electrically generated thermal events. A disadvantage of this approach is that the instrument must be implemented in hardware before it can be evaluated. By developing a scheme to determine the relationship between the measured output and the thermal event in the sample in software, we can evaluate the sensitivity of the output to various geometric design changes, as well as to different construction materials in a computer. This scheme allows the complete design and evaluation of a calorimeter in software.

PROPOSED COURSE: After evaluating several of the Finite Element Simulation packages, we found that most were able to perform only steady-state thermal analyses. In the two packages that could perform transient thermal analysis, the effort involved in getting the physical model into an FES-node model, and in mapping the FES output back into a quantitative form relatable to our physical system, was found to be prohibitive. A model was then developed in SPICE. A SPICE model of the current instrument in use was found to agree with the actual measured response to within 3%. Based on this approach, several new instrument designs are being evaluated in software. In addition, a SPICE model of a convential differential scanning calorimeter (DSC) was developed to investigate the tradeoffs in design between our relaxation-type calorimeter and the DSC. The SPICE model also has the capability of simulating the effect of temperature-dependent phase-transition enthalpies and heat-capacity changes--a capability not present in any FES system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10452-02 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Calorimetric Investigation of Protozoa

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LPD, NIAID (J. Dvorak)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This investigation will determine the feasibility of measuring the metabolic heat produced by protozoa *in vitro*. The first phase of the investigation will involve determining whether the metabolic output of physiologically viable concentrations (10E6 cells/ml) of protozoa can be measured and, if so, developing a protocol for using this measurement as a means of quantitating both the viability of the protozoa and the growth phase (static or exponential).

OBJECTIVE: To develop a calorimetric means of measuring the viability of protozoa that have been exposed to a variety of drugs.

SIGNIFICANCE: If successful, this approach would represent a new mechanism for identifying the effectiveness of new medications for the treatment of various protozoan diseases. Such a calorimetric determination of cell viability would be more economical and fundamentally more sound than the current scheme of visually identifying and counting live cells.

PROPOSED COURSE: A series of preliminary experiments with the protozoa in the calorimeter have revealed that not only is their metabolic heat measureable, but it is also possible to detect the different growth stages (static and exponential) thermally. If it can be confirmed in future experiments, this method will represent a new technique for determining the growth stage, since this is impossible to detect optically. Further experiments are now in progress to refine the experimental protocol for using the calorimeter with the protozoa and confirming the growth-stage measurements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10455-02 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Wavelet Transform Applications to Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR
Michael Unser, Ph.D.	Visiting Fellow	OD, BEIP, NCRR
Michael Vrhel, Ph.D.	NRC Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
MNB, NINDS (S. Sato, S. Schiff); LNP, NINDS (T. Smith)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews
_(b) Human tissues
X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There are many instances of biological data or signals that are by nature nonstationary, e.g., EEG and ECG. Often the goal is to analyze the data or to evaluate the data quantitatively. Because of the data's nonstationary nature, their existence at various size scales, and the desire to locate features in a computationally efficient way, it is important that we use the wavelet transform to process the data appropriately. This transform has the advantage of representing the frequency content of the data and the time at which they occurred. Moreover, the wavelet transform has the ability to extract features at various scales, to enhance edges, and to zoom in on singularities. We have applied the wavelet transform to analyze EEG signals and to detect spikes, oscillations, and the onset of seizures. We are currently investigating its use in estimating the fractal dimension of glial nerve cells to quantitatively measure morphological complexity.

OBJECTIVES: Our goal is to analyze nonstationary biological data and signals, such as EEG and ECG. We want to detect the existence of edges, spikes, oscillation bursts, and other relevant signal features. We also want to reduce the noise in the data with minimal blurring.

METHODS EMPLOYED: Like the Fourier transform, the wavelet transform represents the signal in terms of its frequency content. However, it has the added advantage of being localized in time. It can also detect edges, spikes, and features that are at different time scales. All of these advantages make it the tool of choice for use in the data analysis of nonstationary or transient signals.

MAJOR FINDINGS: We have compared different Fourier methods with the wavelet transform for data analysis of EEG signals, and have shown that the wavelet transform offers certain advantages. We have demonstrated the applicability of the wavelet transform both to spikes and to seizure detection in EEG, but the computational demands have been excessive. Using wavelets associated with particular structures called multiresolutions and using splines, we have reduced the computational demands from several days to several seconds without compromising the accuracy of feature extraction. We have also used the wavelet transform to estimate the fractal dimension of fractal signals. Since images of glial nerve cells are self-similar at different scales, the multiscale structure of the wavelet transform can be used to estimate their fractal dimension.

SIGNIFICANCE: It now appears technically feasible to filter and decompose EEG using wavelet transforms in real time with an ordinary microprocessor. Using this method, it is possible to analyze EEG data accurately. It may even be possible to detect seizures before their onset and to design a pacemaker that will stop them in their earliest stages. In addition, we can measure the fractal dimension of various cells, thus providing quantitative and comparative information for their study and classification. Like the Fourier transform, the wavelet transform can be applied to any biological data (such as ECG signals, images, and 3-D data) for purposes of analysis and evaluation.

PUBLICATIONS: Schiff S, Aldroubi A, Unser M, Sato S. Fast wavelet transform of EEG. Electroencephalogr Clin Neurophysiol 1994;91:442-55.

Unser M. Fast Gabor-like windowed Fourier and continuous wavelet transforms. IEEE Signal Process Lett 1994;1(5):76-9.

Unser M, Aldroubi A. A review of wavelets in biomedical applications. In: Proceedings of the IEEE (in press).

Unser M, Aldroubi A, Schiff SJ. Fast implementation of the continuous wavelet transform with integer scales. IEEE Trans Signal Process. 1994;42:3519-23.

Vrhel MJ, Lee C, Unser M. Fractal dimension estimation using the fast continuous wavelet transform. In: Proc SPIE (Wavelet Applications in Signal and Image Processing III) 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10456-02 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

New Algorithms for Multimodality Image Analysis of the Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR
Chulhee Lee, Ph.D.	Visiting fellow	OD, BEIP, NCRR
Philippe Thévenaz, Ph.D.	Visiting fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

BPB, NIMH (T. Ketter); DICBR, NIAAA (U. Ruttimann, D. Rio)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Functional neuroimaging is a fast-developing area of research aimed at investigating the neuronal activity of the brain *in vivo*. The data for these studies are provided by positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Unfortunately, the analysis of these images is complicated by the variability of the data, by low signal-to-noise ratios, and by limited spatial resolution. Another major difficulty is that the shape and size of the brain varies from one individual to another. The purpose of this project is to develop image-processing algorithms for the quantitative analysis of such data sets. We have designed a general multiresolution procedure for the efficient registration of PET or fMRI volumetric data sets. Our geometrical transformation model allows for translation and general affine transformations (2-D or 3-D), including scaling and rotation. In order to improve the detection of significant differences between subjects, we have developed a method that performs statistical testing in the wavelet domain. The main advantage is that the discriminative information, which is smooth and well localized spatially, becomes concentrated into a relatively small number of coefficients. We have also designed new segmentation procedures to automate the stripping of the brain in MRI, and to detect the corpus callosum. These methods use a priori information on the shape of the objects; this knowledge is typically expressed in the form of connectivity constraints.

OBJECTIVES: There are four main objectives to this research: (1) the (3-D) registration of series of PET or fMRI images (both within and between subjects); (2) the design of quantitative methods for the statistical analysis of these data sets; (3) the segmentation of MRI images for the extraction of brain surface; and (4) correction for various distortions induced by the acquisition process.

METHODS EMPLOYED: Our iterative multiresolution registration procedure represents images in a spline pyramid and uses a coarse-to-fine updating strategy to compute the alignment parameters iteratively. The updates are performed using a variation of the Levenberg-Marquart nonlinear least squares optimization method. The statistical analysis method involves taking the wavelet transform of the difference between aligned group averages, and estimating the within-group variability of the data. A first series of omnibus tests is used to determine the wavelet channels that contain significant differences. Once these channels have been identified, the remaining wavelet coefficients are subjected individually to a follow-up test that localizes the differences spatially. The advantage of this strategy is that the required number of tests is reduced drastically, compared to the traditional approach that considers all pixel values individually.

MAJOR FINDINGS: The multiresolution registration algorithm is capable of very precise image alignment, with subpixel accuracy. It is also much faster and more robust than a comparable single-scale implementation. These findings have been confirmed with real PET and fMRI data; the present algorithm appears to be superior to any other registration method. The wavelet-based statistical method has also been applied to fMRI data, and the results are extremely encouraging. In particular, we were able to demonstrate that the posterior orbitofrontal and pyriform cortices exhibit an increase in activity during the processing of olfactory stimuli.

SIGNIFICANCE: To provide neuroscientists at NIH with new tools for the quantitative analysis of functional images. Since this area of scientific investigation involves very large data sets, it is particularly important to design and promote methods that have a sound statistical foundation.

PROPOSED COURSE: The next step will be to validate our various algorithms and apply them to the processing of 2-D and 3-D data sets. We will also investigate approaches to speed up our registration procedure.

PUBLICATIONS: Lee C, Unser M, Ketter TA. A global approach to multivariate correlation analysis of PET brain images. Proc SPIE (Mathematical Methods in Medical Imaging III) 1994;2299:305-15.

Thévenaz P, Unser M. Efficient geometric transformations and 3-D image registration. In: Proceedings of the International

Conference on Acoustics, Speech and Signal Processing. Detroit: IEEE, 1995;5:2919-22.

Unser M, Thévenaz P, Lee C, Ruttimann UE. Registration and statistical analysis of PET images using the wavelet transform. IEEE Eng Med Biol 1995 (in press).

Lee C, Unser M, Ketter TA. Automatic recognition of corpus callosum from sagittal brain MR images. Proc SPIE 1995 (in press).

Ruttimann UE, Ramsey NF, Hommer DW, Thévenaz P, Lee C, Unser M. Analysis of functional magnetic resonance images by wavelet decomposition. In: Proceedings of the International Conference on Image Processing. Washington, DC: IEEE, 1995 (in press).

Thévenaz P, Unser M. Iterative multiscale image registration without landmarks. In: Proceedings of the International Conference on Image Processing. Washington, DC: IEEE, 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10458-01 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hydrostatic Tissue Clamp

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Stephen Leighton, Sc.D. Mechanical Engineer MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
NINDS (C. Collin); Marine Biological Laboratory (A. Kuzirian)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A hydrodynamic tissue clamp has been conceived, built, and shown to operate successfully. This unique device holds pieces of tissue as small as 1 to 3 cubic millimeters for dissection. It is useful, for example, in isolating particular groups of neurons for tissue culture.

PHS 6040 (Rev. 5/92)

OBJECTIVES: The use of dissecting pins is impractical for delicate tissue fragments because the tissue does not provide enough area to bear on the pins. Forces applied to other areas of the tissue fragment are as likely to tear the tissue adjacent to the pins as they are to cut it, where intended, at the site of the operating tool. This project was begun in order to overcome this problem.

METHODS EMPLOYED: The instrument comprises an open dissection chamber, similar in size and shape to a Petri dish. Most of the area of the bottom of the dish is covered with a layer of gel, to hold dissecting pins for conventional macro-dissection techniques. The volume above the gel layer is filled with a solution compatible with the tissue being dissected (e.g., sterile filtered sea water). Near the center of the dish, a single layer of filter screen lies on top of a filter support above a drain. The tissue for the fine dissection is placed on this screen. The solution is allowed to drain continuously from the dissection chamber into a waste-collection bottle. The dish is kept filled to a constant level via an input tube connected to a constant-level supply system.

MAJOR FINDINGS: The pressure drop created across the screen, typically a few centimeters of water, firmly clamps the tissue in place without concentrated forces. The supply system provides a disturbance-free bath for dissection. In addition to holding the tissue, the constant flow rinses away tiny bits of debris, keeping the area clear for visualization and further dissection. The system can easily be cleaned and sterilized whenever necessary. The dissection chamber fits easily under a dissecting microscope. The bathing solution can be recycled, or fresh solution can be used whenever it is plentiful. We have used this system successfully for dissecting vestibulo-ocular neuron bundles from the nudibranch mollusk *Hermissenda crassicornis*.

PROPOSED COURSE: We expect to continue using the system for the dissection of a variety of tissues. A publication describing the system is pending.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10459-01 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A System to Measure Head Motion Inside an MRI Scanner

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Seth Goldstein, Sc.D.	Chief	MES, BEIP, NCRR

COOPERATING UNITS *(if a*
LCE, NHLBI (P. Jezzard)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)
X _(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
_(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An optical system has been developed to measure the small motions of a person's head during an MRI scan in order to correct the scan and thereby improve the resolution of the image. The system uses two position-sensitive detectors to sense changes sequentially in the positions of three blinking miniature lamps that are mounted on a fixture attached to a bite plate. The computer-controlled instrument computes the six degrees of freedom (three angles and three positions) that are necessary to characterize head movement.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To improve the resolution of MRI scans, it is necessary either to eliminate head motion or to correct for it. Since methods to eliminate head motion have been inadequate to date, the present system has been designed to measure head motion so that corrections can be made to a series of scans taken over an extended period of time.

SIGNIFICANCE: Head motion over the course of a study is a significant problem in certain magnetic resonance imaging (MRI) studies. In particular, studies in which a small signal change is to be measured in some part of the brain resulting from a change in a visible NMR parameter, such as local magnetic susceptibility, can be rendered uninterpretable by even the slightest head motion. Two methods may be used to minimize the deleterious effects of head motion in such time-course imaging studies. The first method is to use software reregistration algorithms that attempt to find a global minimum in a search strategy that minimizes the differences between image data sets acquired at time point t and time point t+dt. These algorithms are often very computationally intensive, and are impractical for use with large data sets. A second method for correction is to use a device that measures the absolute position of the subject's head in the magnet relative to the magnet frame of reference. This method should, in principle, allow a simple rigid-body translation and rotation of the data set to bring the data collected at time t into alignment with with the data collected at time t+dt, and thereby avoid a lengthy computational optimization.

METHODS EMPLOYED: Patient head motion is determined by optically measuring the positions of three blinking miniature lamps affixed to the head, using two position-sensitive detectors (PSDs). These devices sense the x- and y-coordinates of the centroid of the light falling on their photosensitive surface. By using sequentially blinking, bright miniature lights under stable, relatively dark ambient conditions, the PSD output can be related unambiguously to the lamp position. A Macintosh computer running the program Labview performs the sensor data acquisition, as well as the required computations to generate the three angles and three positions required to specify head motion. The lamps are mounted in a small lightweight fixture attached to a bite plate. A set of mirrors, held by a specially designed fixture attached to the patient's pallet, allows the system to look deep inside the magnet bore to take the measurements.

MAJOR FINDINGS: A previously developed system for PET scanners utilizing GPIB-controlled electronics has been replaced with electronics that provides an update every ~0.7 second. The system with the mirrors has sufficient sensitivity to perform the measurements deep inside the magnet with an acceptable data rate. Linearity measured on the bench using motions produced with a mechanical fixture is excellent.

PROPOSED COURSE: The system will shortly be tested inside an MRI scanner, using a special nonmagnetic test fixture to produce all 6 motions. It will then be used with phantoms to test the software for motion compensation, after which trials with patients will be performed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10460-01 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of an AOTF Raman Scattering Microscope

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Seth Goldstein, Sc.D.	Chief	MES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LCP, NIDDK (N. Lewis, L. Kidder)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Mechanical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews

_(b) Human tissues

X _(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are developing a Raman scattering laser scanning microscope. Our novel approach to spectroscopic imaging integrates a commercial optical microscope with a state-of-the-art acousto-optic device (AOTF) and an extremely sensitive low-light-level imaging detector. The instrument is now capable of near-diffraction limited spatial resolution for both Raman and near-infrared (NIR) absorption spectroscopic microscopy, with plans to extend the system into the mid-infrared spectral region.

OBJECTIVES: To improve the performance of a Raman and near-IR light scattering microscope whose feasibility had previously been demonstrated, and to enable it to be used easily as a turnkey instrument with relevant biological samples.

SIGNIFICANCE: Vibrational spectroscopy provides an extremely sensitive and noninvasive probe for examining the structure and dynamic properties of both lipids and proteins in biological materials. In addition to distinguishing between the different components comprising a biological system, it is also readily able to discern reorganizations in these systems as a result of changes in temperature or the presence of drugs, anesthetics, etc. The emphasis of vibrational spectroscopic approaches to the study of biological materials has been on extracting spectroscopic information from samples, with little or no information gleaned about their microscopic structure. We are developing a novel spectroscopic imaging technique that combines the spatial resolving power of optical microscopy with the selectivity of vibrational spectroscopy. Vibrational spectroscopic microscopy is especially powerful because it simultaneously provides the ability to characterize microscopic heterogeneities in samples that can appear spatially homogeneous using conventional optical microscopies or spectroscopically homogeneous from bulk sample spectral analysis. As a result, many different representations of the same sample can be constructed using specific spectral markers to highlight or reveal subtle spatial inhomogeneities in samples that are intractable to other microscopic techniques. In the future, we anticipate that the microscope will prove useful in a host of scientific and technological regimes in which obtaining structural and spectral data on materials simultaneously would be highly advantageous.

METHODS EMPLOYED: The optical train has been redesigned to enhance optical throughput and minimize vignetting and stray light. Wide-field laser illumination was improved by using a vibrating fiber optic to reduce laser speckle and provide a more uniform intensity. A separate light path using two scanning galvanometers was also employed to provide greater flexibility in image acquisition and fields. A high-sensitivity, cooled CCD camera was used to acquire the image under computer control. The AOTF operation and the spectral analysis are also computer-controlled.

MAJOR FINDINGS: The improved system has been designed and built, and is currently being evaluated. As is the usual case with Raman scattering, elimination of stray light and fluorescence is a major problem that will ultimately limit the sensitivity of the instrument.

PROPOSED COURSE: System checkout will be completed. The system can then be used to study problems of interest, including the chemical imaging of cholesterol gradients within retinal rod outer segments, and neurotoxicity problems involving brain slices derived from animals treated with various antitumor and antiaddiction agents. In addition, material studies currently underway include the rapid, noninvasive visualization and characterization of polymer inclusions in human histological tissue sections. If possible the system will be extended into the mid-infrared spectral region.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10461-01 BEI

PERIOD COVERED
April 1, 1995 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
MRS Data Processing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ching-Nien Chen, Ph.D.   Physical Scientist   OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
NINDS (G. DiChiro, G. Tedeschi)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
The goal of this new project is to modify and improve data-processing algorithms for proton spectroscopic data obtained from NMR imagers. Proper phase adjustment needs to be done in order to obtain absorption mode signals. The peak picking algorithm also needs to be revised.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RR 10462-01 BEI

PERIOD COVERED
April 1, 1995 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pulse Programming and Data Processing of MRI on the G.E. Signa System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ching-Nien Chen, Ph.D. Physical Scientist OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
NINDS (G. DiChiro, G. Tedeschi)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The diffusion MRI experiments now being performed on the 2.0-T MRI system need to be ported to the G.E. Signa system in order to do human studies. Since the two imaging systems differ in hardware and software, the software packages (including data collection, pulse generation, and data processing) must be rewritten. For experiments involving short relaxation times, different algorithms need to be implemented or devised.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10463-01 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Heart-Rate Variability in Endotoxemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Alec Eidsath, Ph.D. Chemical Engineer MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
CCM, CC (P. Godin)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Normal, physiologic heart-rate variability (HRV) is modulated by certain diseases. We infused endotoxin into volunteers to model early clinical sepsis, and analyzed HRV by comparing a conventional technique (Std Dev) with approximate entropy (ApEn).

PHS 6040 (Rev. 5/92)

OBJECTIVES: To determine whether approximate entropy analysis can be used to analyze the HRV of early clinical sepsis.

METHODS EMPLOYED: We administered endotoxin (4 ng/kg i.v.) and also vehicle alone (in random order, 1 week apart) to each of 8 normal volunteers, 4 of whom also received Ibuprofen (Ibu, 2400 mg po total). The data was digitized and analyzed under the control of a "virtual instrument" written in the LabView programming language. An ECG was continuously acquired and digitized at 200 Hz. The 1st 1000 R-R intervals of each of 8 hourly epochs were analyzed. The power spectrum of the instantaneous heart rate was also analyzed.

MAJOR FINDINGS: Endotoxin produced fever and tachycardia, and reduced HRV as estimated by both approximate entropy ($p<0.0002$) and Std Dev ($p<0.0001$). However, normalizing changes in ApEn to those of Std Dev demonstrated an additional, increased regularity component of HRV undetected by Std Dev ($p<0.03$). Administration of Ibu, which obviated all other clinical manifestations of endotoxin, did not affect the reduction in HRV by either measure.

SIGNIFICANCE: Decrement in HRV may be a useful early sign of human sepsis because it is not confounded by ibuprofen therapy. Approximate entropy detects the regularity dimension of endotoxin-reduced HRV, which is unrecognized by Std Dev and other conventional moment statistics.

PROPOSED COURSE: The HRV data has been analyzed. We are now in the process of analyzing the respiratory data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10464-01 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Acoustic and Visual Communication in a 4-Tesla MRI Apparatus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

VSL, NIDCD (A. Braun); Walter Reed Army Medical Center (T. Balkin, N. Wesensten)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects
(a1) Minors
(a2) Interviews

(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Magnetic resonance imaging provides a modality for researchers to study the regions of the brain associated with the speech disorder of stuttering. Without an auditory stimulus or feedback, persons who stutter will not manifest the disorder. Traditional devices for voice communication all use ferromagnetic components in their construction. Introduction of metallic components into high, uniform magnetic fields, such as the 4-Tesla field associated with a magnetic resonance imager (MRI), is undesirable for two reasons: First, the presence of ferromagnetic materials presents a safety hazard; and second, ferromagnetic materials disturb the magnetic field's uniformity, which is essential to the production of high-quality scans. A non-ferromagnetic microphone based on fiber-optic technology has been developed to permit communication between the patient and the researcher. The use of pneumatic transducers as a mechanism for providing auditory feedback to the patient is being evaluated.

OBJECTIVES: To develop a device that will provide both auditory feedback to the patient and communication with the researcher. The device must be capable of operating in the high magnetic field of an MRI scanner, and it must not affect the uniformity of the MRI field. The device must also provide satisfactory auditory feedback in the presence of the high ambient acoustical noise associated with the operation of the MRI scanner.

METHODS EMPLOYED: A microphone based on fiber-optic technology has been developed. The cleaved faces of two 200-µ-diameter optical fibers are positioned end to end, with a small (i.e., approximately 250 µ) separation between the cleaved faces. An opaque knife edge, mounted on a membrane diaphragm, is inserted into the gap in such a way that vignetting of the fiber face end-to-end overlap occurs. One of the two optical fibers carries light from a stable light source. The light that transverses the gap is collected by the second optical fiber, the distal end of which is alligned with a photodiode. Acoustic signals cause deformation of the membrane diaphragm, which produces a modulation of the light transmission across the gap. The photodiode's output after processing is fed into an audio amplifier, which drives a loudspeaker.

A prototype device was constructed on an optical bench to demonstrate the proof of the principle. A second, hand-held device, consisting solely of nonferromagnetic materials, has also been constructed and is being evaluated.

Thirty feet of pressure tubing were routed through the bore of the MRI scanner, and an acoustic signal was introduced at one end. The quality of the signal at the output end was evaluated during an MRI scan with gradient switching.

MAJOR FINDINGS: Both the prototype and the hand-held versions of the device produce acceptable fidelity for voice communication. A normal speaking voice is detected with the diaphragm positioned within a few inches of the patient's mouth. The optical fibers carrying the light source and the modulated light signal are immune to motion artifacts associated with either direct (i.e., patient-induced) movement or involuntary movement of the fibers due to the mechanical vibrations associated with the gradient field switching during the scanning process. The device has not been tested within the bore of the MRI magnet, nor has the sensitivity of the device to the noise present during a scan been determined.

The pneumatically driven tubing produced clear audio signals, and was immune from the noise caused by the field switching.

SIGNIFICANCE: Communication with patients inside the bore of a high-field MRI scanner is a problem that has not been addressed by the commercial marketplace. This nonmagnetic microphone coupled to pneumatically driven headphones offers a solution to this need. In the research environment (for example, in studies of the speech disorder of stuttering), this device provides the patient with the necessary audible feedback. The acoustical noise level inside the MRI scanner requires the use of ear protection, preventing the patient from receiving this audible feedback and from receiving instructions from the researcher.

PROPOSED COURSE: The device will be evaluated inside the magnetic field of the MRI scanner to assess its performance and its ability to reject the acoustical noise associated with the switching gradients. A version for clinical use will be fabricated once the final performance criteria have been established and the testing has been performed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10465-01 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fluorescence Analysis of Inter- and Intracellular pH and Membrane Potential

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
Horace E. Cascio, BSEE	Electrical Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LCB, NHLBI (R. W. Hendler, A. K. Mukhopadhay)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A fiber-optic-based instrument was developed to make simultaneous measurements of inter- and intracellular pH and membrane potential of vesicles containing bacteriorhodopsin in the presence of high-intensity actinic light. Fluorescent probes are incorporated into the membrane or intracellular space, and are excited at their characteristic wavelength. Most frequently, pyrinine and oxanol V or VI correlated independently with pH and ion-selective electrodes.

OBJECTIVES: To develop a methodology for assessing the pH and membrane potential of vesicles containing bacteriorhodopsin during stimulation by high actinic light levels.

METHODS EMPLOYED: A specially designed reaction chamber was provided with access ports for two fiber-optic probes, a pH electrode, and an ion-sensitive electrode (thiocyanate). One port contains the optical fiber assembly, consisting of nineteen 600-μ fused silica fibers arranged in a symmetric bundle. The distal end is immersed in the reaction mixture, and was encased in a sleeve that accommodated an observation window. The proximal end of the fiber is separated into two excitation and two emission arms. The sleeve is necessary to prevent capillary action from trapping the contaminants from one reaction and releasing them in a subsequent reaction. Appropriate 10-nm bandpass filter pairs (for example, the detection of oxanol requires 580-nm excitation and 650-nm emission) are positioned at the end of each arm. Excitation is provided by a 50-W tengsten-halogen lamp, chopped at 1Khz by a 50% duty-cycle rotating-blade shutter, and the fluorescent signal from the reaction solution is sampled by the emission fibers and carried to an integrated photomultiplier/pre-amplifier photodetector. The photomultiplier output is demodulated, further amplified, and provided with adjustable DC-offset and time-constant controls.

The actinic light is provided by a 100-W quartz-halogen lamp situated at the focus of a parabolic reflector. The collected light is imaged on the reaction vessel after transmission through a 10-cm water filter to remove unwanted heat. Masking prevents the actinic light from impinging directly on the magnetic stirrer, causing high-intensity scattered light. A photodiode, incorporated into the holder for the reaction vessel, monitors the intensity of the actinic light.

MAJOR FINDINGS: Fiber-optic technology has proven to be an effective means of measuring the intracellular pH and membrane potential of vesicles containing bacteriorhodopsin. The effective sample volume is located within a few millimeters of the end-face of the fiber-optic probe. Modulation of the monitoring excitation light enables detection of the weak fluorescent signal in the presence of the very high background light from the DC-level, actinic white-light source. Two significant sources of noise are the scattered actinic light from the stirring bar, and noise from the vesicle suspension. A major reduction in the former is accomplished by shielding the stirring bar from direct light with residual fluctuations caused by indirect reflection from the vessel walls. This and the vesicle scatter are minimized by selecting a long time-constant (0.1 sec) in conjunction with the 1-Khz chopping frequency.

SIGNIFICANCE: This instrumentation enables simultaneous measurements of intracellular pH and membrane potential, which was not possible using electrode technology. The measurements can also be made during illumination of the bacteriorhodopsin at high actinic light level, which affects electrode readings.

PROPOSED COURSE: To further develop and refine the instrumentation for application in studying the bacteriorhodopsin photocycle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10466-01 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Muscle Cell Electrical Stimulation to Study Hypertrophic Cardiopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas J. Pohida, MSEE	Electronics Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

CB, NHLBI (N. Epstein)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews

(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease characterized by an increase in ventricular mass in the absence of another cause for cardiac hypertrophy. Approximately 15% of familial cases are caused by one of more than 37 distinct point mutations in the Beta myosin heavy chain (BMHC) gene. This gene has been shown to be expressed in slow skeletal muscle fibers as well as in the heart. Myosin extracted from skeletal muscle of these HCM patients demonstrates abnormal function in a variety of *in vitro* assays. Furthermore, histological examination of these biopsies reveals the presence of skeletal muscle hypertrophy and the absence of mitochondria from the center of many of the type-I fibers, a pattern resembling central-core disease.

Studies in other labs have shown that primary cultures of normal animal skeletal myoblasts undergo hypertrophy when subjected to an alternating electrical field. Myocytes expressing the BMHC gene mutations are predisposed to hypertrophy in the patient. Thus, it should be possible to study the molecular concomitants of hypertrophy induced by an electrical field in myoblasts from normal individuals as well as in myoblasts obtained from HCM patients with identified BMHC mutations.

OBJECTIVES: To develop a programmable cell-stimulation system optimized to facilitate these and other experiments.

METHODS EMPLOYED: The system will include an embedded PC to provide flexibility in the stimulation procedure. Parameters that will be programmable will include voltage, current, and pulse rate. The system will communicate with an external PC so that various stimulation sequences can be automated.

SIGNIFICANCE: Phenotypes in the normal and patient cell lines, which are induced by stimulation, will be studied at the molecular level and should shed light on the process of hypertrophy resulting from mutations in the BMHC gene.

PROPOSED COURSE: Skeletal muscle cell lines from selected patients are being developed. The cell-stimulation system being designed and developed by the EEES will be used to subject these cells to an electric field.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10467-01 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Spectrofluorometric Measurement of the Critical Bilayer Assembly Temperature T*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas L. Talbot, M.S.	Mechanical Engineer	ACES, BEIP, NCRR
Courtney Mudd, Ph.D.	Biomedical Engineer	ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LPB, NIAMS (N. Gershfeld); LCP, NIAMS

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues X (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study of the molecular mechanisms used by cells for the assembly of membranes is the principal focus of the instrumentation being developed in this project. The lipid bilayer of membranes has been shown to self-assemble at a critical point, T*, which is identical to the physiological temperature, previously measured by equilibrium thermodynmamic methods. Evaluation of T* under equilibrium conditions has been limited by the intrinsic slowness of the bilayer assembly processes at the critical point. Thus a measurement of T* is limited by the speed at which equilibrium is reached, and may take several weeks to complete. We have developed a scanning method for measuring T* that obviates the need to wait for equilibrium and allows a complete T* measurement in several hours. This method is now being implemented for large scale measurement of membrane samples for the detection of lipid-based metabolic defects in neurological tissues.

METHODS EMPLOYED: The method consists of applying a shearing force to an aqueous suspension of liposomes containing a fluorescent dye at self-quenched concentrations. The liposomes are composed of the complete lipids extracted from the membranes of a tissue. At a threshold value of the shearing force, the bilayers rupture. Dye leaks into the external solution and is diluted by a factor of 10^5. At this lower concentration, the dye is no longer quenched; therefore, it fluoresces. The magnitude of the shear stress sufficient to rupture the bilayer is a function of temperature, decreasing by approximately 25% at T* from the rupture shear stress at temperatures below and above T*.

MAJOR FINDINGS: The value of T* obtained by this method agrees with that obtained by equilibrium methods. Thus, the shearing forces do not change the fundamental molecular process entailed in the formation of the critical bilayer state, but only accelerate the processes that normally occur. We have made this property the basis of a scanning method in which we apply a shearing force, fixed at a value sufficient to cause leak at T*, but too low to rupture the bilayers at temperatures below and above T*.

PROPOSED COURSE: In order to study the phenomena described above, precise temperature regulation of the samples under investigation is imperative. A four-position sample holder has been designed and fabricated that incorporates Peltier devices to regulate the temperature of the liposomes contained in standard 1-cm path length cuvettes. An appropriate temperature-control unit is currently under construction. In addition, a rotating disk within a cylinder that will fit into the cuvette is under study to impart a well-defined and reproducible shear stress upon the liposomes.

This device will be interfaced to a personal computer that will provide the temperature set points for the samples, control the rotating disk, display the temperature in each of the four cells, and display a fluorescence vs. temperature graph for each of the four samples. The fluorescence measurements will be obtained with a Perkin Elmer LS50B spectrofluorometer that will be controlled by custom software via the integral RS 232 port. Simple programs have demonstrated the feasibility of this approach, and the entire system, integrating the spectrofluorometer, the temperature-control unit, and the personal computer, is under development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10470-01 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure of Clathrin Cages by STEM and Metal Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR
Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR
Megan Jatko	Summer Student	OD, BEIP, NCRR
Alim Somani	Summer Student	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
PSL, DCRT (A. Jin, R. Nossal); LCB, NHLBI (K. Prasad)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Methods are being established to characterize the structure and assembly of clathrin triskelions and clathrin cages *in vitro* to help our understanding of the energetics of receptor-mediated endocytosis. Preparations of the clathrin macromolecular assemblies in various buffers were adsorbed onto thin carbon substrates supported on EM grids, then rapidly frozen in liquid ethane at -180°C. Specimens are either cryotransferred into a high-resolution field-emission STEM for measurement of molecular-weight distributions, or cryotransferred into a vacuum chamber for freeze-etching and platinum/carbon shadowing. It has been necessary to determine the conditions for freeze-drying and metal shadowing to achieve optimal resolution. The metal replicas are imaged digitally in a transmission electron microscope equipped with a slow-scan cooled CCD camera controlled by a Power Macintosh computer. Digital electron microscopy has the advantage of providing immediately accessible data without the need for a dark room.

OBJECTIVES: To characterize the structures of clathrin cages and triskelions *in vitro* by scanning transmission electron microscopy and freeze-etch metal replication.

METHODS EMPLOYED: Clathrin and tobacco mosaic virus (TMV) as a mass calibration were adsorbed onto thin carbon films supported over EM grids, then rapidly frozen in liquid ethane. Some specimens were cryotransferred into a VG Microscopes HB501 STEM, where they were freeze-dried and imaged at low dose to determine their molecular-weight distributions. Other samples were cryotransferred into a Cressington CFE40 vacuum chamber for freeze-etching and rotary shadowing with platinum/carbon from an electron-beam gun oriented at approximately 20° to the horizontal. The coated samples were imaged digitally using a slow-scan CCD camera on a 200-keV transmission electron microscope.

MAJOR FINDINGS: It has been possible to quantify the total mass distributions of the clathrin cages by annular dark-field imaging in the STEM; and from the known mass of the clathrin triskelions, it is possible to infer the distribution of subunits that make up the cages. Conditions for metal shadowing with the Cressington CFE40 vacuum chamber are being established, and three-dimensional views of these structures are expected to provide further information about their assembly.

SIGNIFICANCE: Clathrin complexes are involved in receptor-mediated endocytosis and in the formation of coated vesicles and coated pits. Structural work on these proteins may help elucidate the deformation properties of the triskelions and the energetics of their assembly into cages.

PROPOSED COURSE: To improve the quality of the specimen preparation and microscopy. To develop three-dimensional stereo imaging of the metal-shadowed clathrin cages, using the digital-microscopy setup on the Hitachi H700H TEM.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10471-01 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Application of a Post-Column Energy Filter

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR
Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*

LSB, NIAMS (F. P. Booy, A. C. Steven); LN, NINDS (S. B. Andrews); Gatan Inc. (O. L. Krivanek)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An advanced energy-filter on a H700H cryo-electron microscope is being assessed for digital imaging and analysis of biological structures at high sensitivity. The filter is equipped with a YAG-coupled, cooled, slow-scan CCD camera, and is entirely computer-controlled. The detective quantum efficiency of the CCD is greater than 0.7 for incident fluxes from 10 electrons per pixel to 10000 electrons per pixel. The low-intensity performance is improved relative to that of a cooled photodiode array because of its much lower read-out noise. The high-intensity performance is improved because the CCD images can be accurately gain-normalized. The normalization is made possible because the small pixels of the CCD (24 microns square) are smaller than the typical spatial variation of the incoming signal. It has been demonstrated that spectra can be acquired in the normal mode that contain as many as 100 million fast electrons per channel, corresponding to statistics at the 0.01% level. This is expected to reduce detection limits for elemental analysis of biological specimens to a point that is even lower than can be achieved with a photodiode array detector. The use of the imaging filter is also being explored for the acquisition of low-dose micrographs of beam-sensitive specimens.

OBJECTIVES: To assess the use of a post-column energy filter on a cryo-electron microscope for digital imaging and electron energy-loss spectroscopy of biological specimens.

METHODS EMPLOYED: A post-column imaging filter, optimized for biological microscopy and spectroscopy, has been used with a 200-keV transmission electron microscope. The filter and associated cooled slow-scan CCD array is controlled by a Power Macintosh computer, which enables both spectroscopy and digital microscopy to be performed at 200 keV. To test imaging of frozen-hydrated specimens, it was necessary to modify the design of our liquid nitrogen-cooled anticontaminator blades in order to reduce ice contamination.

MAJOR FINDINGS: The stability of the energy filter on the Hitachi H700H electron microscope was found to be satisfactory both at 100 keV and at 200 keV of beam energy. The cooled CCD detector provided spectra in normal mode (i.e., without differentiation) that had high detective quantum efficiency and that could be gain-corrected with high accuracy. This demonstrates the advantage of CCD detectors over the photodiode array for EELS analysis. The detector was also shown to provide high-resolution digital energy-selected images at low electron dose. The modification to the anticontaminator blades successfully eliminated ice contamination on the specimen.

SIGNIFICANCE: The energy-filtering transmission electron microscope provides complementary capabilities to the dedicated STEM instrument equipped with parallel-detection EELS. The energy-filter, combined with a high-performance CCD detector, allows collection of million-pixel images in short times, while the STEM provides extremely high sensitivity for elemental analysis with smaller numbers of pixels. This work could help to establish which of these two very different instruments is better suited to answer particular biological problems.

PROPOSED COURSE: (1) To compare the performance of the charge-coupled-device (CCD) detector on the energy-filtering TEM with the photodiode-array detector on our VG Microscopes STEM in terms of sensitivity for elemental microanalysis. (2) To demonstrate the feasibility of quantitative imaging of phosphorus in nucleic acid/protein complexes (e.g., viruses and ribosomes). 3) To demonstrate whether the imaging filter can provide significant enhancement in resolution and contrast for cryomicroscopy of frozen-hydrated cells and isolated macromolecular assemblies. (4) To investigate new approaches for mapping multiple elements (e.g., phosphorus and sulfur) in rapidly frozen, cryosectioned cells.

PUBLICATIONS: Krivanek OL, Kundmann MK, Trevor C, Leapman RD. Improved PEELS performance with an imaging filter. In: Proceedings of the 29th annual MAS meeting. 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 RR 10472-01 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Low-Dose Energy-Selected Electron Microscopy with an Omega Filter

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
LN, NINDS (S. B. Andrews)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have investigated the potential of using an in-column omega filter in a conventional transmission electron microscope for elemental mapping of biological structures at low dose. Digital images were collected at a beam energy of 100 keV by means of a 1024 x 1024-pixel, phosphor-coupled, cooled slow-scan CCD camera on a Zeiss 912 electron microscope. Frozen-hydrated specimens of herpes simplex virus (vitrified by plunging into liquid ethane) were cryotransferred and freeze-dried before imaging in the region of the phosphorus L-edge at energy losses of around 130 eV. These digital micrographs were recorded from large numbers of viruses at a dose of less than 1000 electrons per square nanometer. Resulting noisy images were corrected for read-out noise and pixel-gain variations before cross-correlating, summing, and removing the noncharacteristic background image extrapolated from the pre-edge region of the EELS spectrum into the post-edge region. In this way, it was possible to build up an averaged projected distribution of phosphorus, i.e., nucleic acid, in the core of the herpes simplex capsid.

OBJECTIVES: To map the distributions of phosphorus and other elements in biological structures at high spatial resolution and low electron dose (less than 1000 elecrons per square nanometer) by averaging over many identical objects.

METHODS EMPLOYED: The instrumentation consisted of a Zeiss 912 transmission electron microscope, operating at a beam energy of 100 keV and equipped with an omega-filter energy spectrometer and a 1024 x 1024-pixel, phosphor-coupled, cooled slow-scan CCD camera. Frozen-hydrated specimens of herpes simplex virus were cryotransferred and freeze-dried at -100°C. Digital images were recorded at zero energy loss and at energy losses in the region of the phosphorus L-edge at 130 eV. These data were processed by (i) subtracting read-out noise, (ii) correcting for detector gain, (iii) correlating and adding the noisy inelastic images, and (iv) extracting the characteristic core-edge image of phosphorus by removing the background-extrapolated image.

MAJOR FINDINGS: It was demonstrated that the read-out noise of the cooled CCD detector was equivalent to approximately 2 fast electrons per pixel. Energy-selected images of the herpes simplex virus recorded at low electron dose in the region of the phosphorus L-edge contained on average only about one fast electron per pixel. By aligning and summing these noisy images from many identical structures, it was possible to obtain the distribution of nucleic acid in the core of the virus. By contrast, control images from empty capsids showed no detectable phosphorus.

SIGNIFICANCE: As a result of several recent developments, EELS is emerging as a useful technique for analyzing beam-sensitive biolgical specimens. There are two complementary approaches to acquiring energy-loss information at high spatial resolution: The field-emission STEM offers detailed spectral information and high sensitivity, while the energy-filtering TEM has a significant advantage when data must be collected from large numbers of pixels. Preliminary experiments demonstrate the feasibility of low-dose energy-loss imaging of rapidly frozen biological specimens using the weak signal in the core loss spectrum at around 130 eV. As one example, this technique could be useful for mapping nucleic-acid distributions in viruses and other protein/nucleic-acid complexes.

PROPOSED COURSE: To average energy-selected images from large numbers of virus particles in order to improve the signal-to-noise ratio in the phosphorus maps. To test the feasibility of recording phosphorus distributions from hydrated viruses, and to improve methods for cross-correlating and processing the noisy images.

PUBLICATIONS: Andrews SB, Gallant SB, Sun SQ, Leapman RD. Low-dose energy filtering in biology. In: Proceedings of the 29th annual MAS meeting. 1995 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10473-01 BEI

PERIOD COVERED
October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Positron Emission Mammography Scanner

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen Markowitz, M.E.E.	Electrical Engineer	ACES, BEIP, NCRR
Burton Chidakel, B.S.	Instrument Maker	BEIP, NCRR

COOPERATING UNITS *(if any)*
LDRR, OD (I. Weinberg)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section, CCIS

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

(a) Human subjects
  (a1) Minors
  (a2) Interviews
(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A positron emission mammography (PEM) instrument is being developed to be used as a clinical and research tool for the diagnosis and planning of treatment for breast cancer. Dedicated instrumentation for PEM was implemented in a standard x-ray mammography unit by positioning two gamma cameras, one above and one below a compressed breast. The radiotracer fluorodeoxyglucose (FDG) is administered to the patient. FDG is known to concentrate in breast tumors, thus making them detectable in positron emission tomagraphy (PET) systems. FDG emits gamma rays, which are detected by the gamma cameras. Each camera has a last-dynode output connected to a coincident circuit for event trigger. The data from each camera is analyzed and placed in memory. Images of the distribution are obtained in real time and displayed. The system parameters include: (1) real-time image capability, so that a 1-cm hot spot can be seen within 30 microseconds; (2) resolution of a 1-cm-diameter hot spot in a phantom, so that a hot spot-to-background ratio will be 8:1 or greater; and (3) a high singles rate in the range of 100 KHz with a minimum separation of pulses, to avoid overlap, in the 1.0- to 1.5-sec range. The instrument development includes the design and fabrication of the detectors, signal-processing and data-acquisition electronics, and real-time programming for acquisition, display, and storage of events.

OBJECTIVES: The instrument will be complementary to conventional mammography, standard PET, and magnetic resonance imaging systems. The unit will have an improved sensitivity and specificity, primarily for the detection of breast carcinoma, over conventional mammographic units, and will utilize dual high-resolution scintillation cameras. To minimize cost and improve access, the device will be designed as an accessory to existing conventional mammomgraphy instruments.

METHODS EMPLOYED: The PEM unit consists of two gamma-ray detector modules, a modified conventional mammographic unit, Computer Automated Display and Control Modules (CAMAC) and Nuclear Instrumentation Modules (NIM) for signal processing, and a Macintosh computer for analysis, display, and storage of data

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The detector modules are identical. Each contains a Hammamatsu R3941-05 photomultipier (PMT) with a useful field of view of about 65 mm x 55 mm; 1-cm-thick slabs of Bismuth Germanate (BGO) scintillator crystal coupled to the PMT with preamplifiers for x and y grid position of each event; and a last-dynode output preamplifier for event trigger. Event capture is obtained when an event occurs, within a 50-nsec window, on both detectors. The event capture utilizes constant fraction discriminators and fast logic units. The NIM include fast amplifiers, constant fraction discriminators, and fast logic units. The CAMAC modules include Charge ADCs (FERA), dual port memory, SCSI, and stepping-motor controllers. The data via the SCSI interface will be transmitted to a Macintosh computer. The real-time display, analysis, and storage of the data is accomplished with the KMAX application package and the C programming language. The system software performs the following operations: (1) calculates event position; (2) corrects for spatial, energy, efficiency, and last-dynode nonlinearity; (3) has a specific and predetermined angular acceptance; (4) performs image reconstruction by the back projection algorithm; and (5) stores and displays data.

SIGNIFICANCE: Conventional mammographic techniques are not ideal for detecting cancers in women with fibrocystic tissue, for determining the presence of axillary spread of tumor, or for monitoring response to chemotherapy. This system is intended to provide this information. Work in the field of PET has demonstrated that information about metaboloic and molecular biology of breast tumors can be provided noninvasively. The instrument will be a low-cost, high-resolution system, compared to standard PET systems.

PROPOSED COURSE: System testing on the instrument will continue as part of the debugging procedure. An emission mammography facility is under construction at the NIH Clinical Center. The facility will incorporate PEM-detector modules in a prone mammography unit that will have stereotactic biopsy and spot digital x-ray mammography capabilities. Protocols are being developed for the facility that will involve patients with

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advanced breast cancer, as well as asymptomatic women who are considered to have increased risk for developing breast cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10474-01 BEI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ganzfeld Stimulator for Clinical Electroretinography

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen Markowitz, M.E.E.	Electrical Engineer	ACES, BEIP, NCRR
Burton Chidakel, B.S.	Instrument Maker	BEIP, NCRR

COOPERATING UNITS *(if any)*

CB, NEI (R. Caruso)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section, CCIS

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.25	PROFESSIONAL: 0.15	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

X-(a) Human subjects
(a1) Minors
(a2) Interviews
(b) Human tissues
(c) Neither

Summary of Work (Use standard unreduced type. Do not exceed space provided)

The Clinical Branch of the National Eye Institute asked us to develop an electroretinogram (ERG). This instrument records the field electrical potential generated by the retina and elicited by a visual stimulus. Electroretinography is a noninvasive technique used in the diagnosis and management of diseases of the retina.

To elicit an ERG, an appropriate stimulator must be used to deliver a visual stimulus to the retina. In clinical electroretinography, this stimulus is usually a brief flash of light with known luminance and spectral composition. The clinically relevant parameters of the ERG depend, among other variables, on the magnitude of the retinal area stimulated by light. The ideal stimulus for most clinical applications illuminates the entire retina uniformly. This requires an extended source of uniform luminous intensity covering the entire visual field. The instrument being designed is called a Ganzfeld (full-field) stimulator; it operates using the principle of an integrating sphere.

ERG waveforms vary with the characteristics of the light stimulus and the adaption state of the retina. For this reason, a Ganzfeld dome stimulator should allow the control of luminance and spectral composition of both stimulus and adapting background. A convenient way to achieve this end is to interpose neutral density filters (to vary spectral composition) between the light source and the Ganzfeld dome.

OBJECTIVES: Various components of the ERG are elicited by the onset of a light stimulus (on-ERG) and by its offset (off-ERG). Two clinically relevant components of the on-ERG are the a-wave, which is generated by the photoreceptor cells (late receptor potential), and the b-wave, which is generated by the retinal glial cells (Muller cells). The most conspicuous component of the off-ERG is the d-wave, largely produced by the offset of the late receptor potential. The most widespread stimulus for clinical electroretinography is a short light flash, generated by a gas discharge tube. Due to the brief stimulation duration (10 to 20 microseconds), there is a partial overlap of on- and off-ERG components. To isolate the off-ERG components, a longer stimulus duration (10 to 100 ms) is essential. These longer stimuli can be generated using a high-intensity continuous light source, such as a quartz-tungsten-halogen lamp, driven by a constant-current power supply. The onset and offset of the stimulus can be controlled with a precision shutter.

METHODS EMPLOYED: The unit contains a control chassis, a sphere with an opening for the subject's head, and a Nicolet Pathfinder monitoring unit for amplication, display, and computer storage. The sphere contains a mounted quartz-halogen lamp assembly with an electronic shutter, a fixation light, and a background light assembly. The time for shutter opening is set on the control chassis. The control chassis contains the electronics and the interface for all component assemblies.

SIGNIFICANCE: The instrument is valuable in the evaluation of retinal and choroidal dystropies and secondary degenerations, vascular retinopathies, retinal and choroidal diseases, and toxic retinopathies.

PROPOSED COURSE: The unit has been completed, and preliminary clinical use has been initiated. Design modifications will be accomplished after initial patient testing.

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